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(54) Title: TREATMENT OF METABOLIC DISORDERS WITH A TNF RECEPTOR FAMILY MEMBER (FRADJ AND/OR CRYPTIC) AGONISTS OR ANTAGONISTS

(57) Abstract: The invention concerns FRADJ and/or CRYPTIC polynucleotides and polypeptides, to screening methods for identifying ligands of FRADJ and/or CRYPTIC, and to their use in the treatment of FRADJ and/or CRYPTIC-related disorders.

TREATMENT OF METABOLIC DISORDERS WITH A TNF RECEPTOR FAMILY MEMBER (FRADJ AND/ OR CRYPTIC) AGONISTS OR ANTAGONISTS

Field of the Invention

The present invention relates to the field of metabolic research, in particular the discovery of compounds effective for reducing body mass and useful for treating obesity-related diseases and disorders. The present invention additionally relates elsewhere to the field of metabolic research, in particular the discovery of compounds effective for increasing body mass and useful for treating disorders associated with excessive weight loss. The obesity-related diseases or disorders envisioned to be treated by the methods of the invention include, but are not limited to, hyperlipidemia, atherosclerosis, diabetes, and hypertension. The disorders associated with excessive weight loss and envisioned to be treated by the methods of the invention include, but are not limited to, cachexia, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, and anorexia.

The present invention is directed to polynucleotides encoding FRADJ and/or CRYPTIC polypeptides and fragments thereof. The invention also concerns polypeptides encoded by the FRADJ and/or CRYPTIC polynucleotides and fragments thereof. The present invention also relates to recombinant vectors, which include the polynucleotides of the present invention, particularly recombinant vectors comprising a FRADJ and/or CRYPTIC regulatory region or a sequence encoding a FRADJ and/or CRYPTIC polypeptide, and to host cells containing the polynucleotides of the invention, as well as to methods of making such vectors and host cells. The present invention further relates to the use of these recombinant vectors and host cells in the production of the polypeptides of the invention. The invention further relates to antibodies that specifically bind to the polypeptides of the invention and to methods for producing such antibodies and fragments thereof. The invention also provides for methods of detecting the presence of the polynucleotides and polypeptides of the present invention in a sample, methods of diagnosis and screening of abnormal FRADJ and/or CRYPTIC expression and/or biological activity, methods of screening compounds for their ability to modulate the activity or expression of FRADJ and/or CRYPTIC and uses of such compounds. In particular, the invention provides for methods of identifying and using ANTAGONISTS and AGONISTS of FRADJ and/or CRYPTIC activity. The invention further relates to the use of FRADJ and/or CRYPTIC agonists for treatment and/or prevention of obesity-related disorders, and to the use of FRADJ and/or CRYPTIC antagonists for treatment and/or disorders associated with excessive weight loss.

Background of the Invention

The following discussion is intended to facilitate the understanding of the invention, but is not intended nor admitted to be prior art to the invention.

Obesity is a public health problem that is serious, widespread, and increasing. In the United States, 20 percent of the population is obese; in Europe, a slightly lower percentage is obese (Friedman (2000) Nature 404:632-634). Obesity is associated with increased risk of hypertension, cardiovascular disease, diabetes, and cancer as well as respiratory complications and osteoarthritis (Kopelman (2000) Nature 404:635-643). Even modest weight loss ameliorates these associated conditions.

While still acknowledging that lifestyle factors including environment, diet, age and exercise play a role in obesity, twin studies, analyses of familial aggregation, and adoption studies all indicate that obesity is largely the result of genetic factors (Barsh et al (2000) Nature 404:644-651). In agreement with these studies, is the fact that an increasing number of obesity-related genes are being identified. Some of the more extensively studied genes include those encoding leptin (*ob*) and its gene (*db*), pro-opiomelanocortin (*Pomc*), melanocortin-4-gene (*Mc4r*), agouti protein (A^y), carboxypeptidase E (*fat*), 5-hydroxytryptamine gene 2C (*Htr2c*), nescient basic helix-loop-helix 2 (*Nhlh2*), prohormone convertase 1 (*PCSK1*), and tubby protein (*tubby*) (rev'd in Barsh et al (2000) Nature 404:644-651).

Recently it was shown that particular carboxyl-terminal fragments of the full-length ACRP30 (mouse) and APM1 (human) polypeptides have unexpected effects *in vitro* and *in vivo*, including utility for weight reduction, prevention of weight gain, and control of blood glucose levels (Fruebis et al (2001) Proc Natl Acad Sci USA 98:2005-10). The effects of ACRP30 fragment administration in mammals also include reduction of elevated free fatty acid levels including elevated free fatty acid levels caused by administration of epinephrine, *i.v.* injection of "intralipid", or administration of a high fat test meal, as well as increased fatty acid oxidation in muscle cells, and weight reduction in mammals consuming a normal or high fat/high sucrose diet.

Summary of the Invention

The instant invention is based on FRADJ and/or CRYPTIC as receptor for LIGANDS that mediate effects, including utility for weight reduction, prevention of weight gain, and control of blood glucose levels in humans and other mammals. These effects in mammals of FRADJ and/or CRYPTIC engagement by LIGAND also include reduction of elevated free fatty acid levels including elevated free fatty acid levels including elevated free fatty acid levels caused by administration of epinephrine, *i.v.* injection of "intralipid", or administration of a high fat test meal, as well as increased fatty acid oxidation in muscle cells, and weight reduction in mammals consuming a normal or high fat/high sucrose diet.

More specifically, the present invention is directed to FRADJ and/or CRYPTIC to which LIGAND binds and through which LIGAND mediates said effects.

FRADJ and/or CRYPTIC is a cell surface transmembrane polypeptide belonging to the Tumor Necrosis Factor Receptor Super-Family (TNFRSF). Ligands of the TNFRSF include APM1. The extracellular domain of TNFRSF2 polypeptide, a representative member of this super-family of related receptors, binds specifically with a high affinity to the globular C1q-homology region of APM1 (see Example 18). (APM1 polypeptide fragments comprised of all or part of the APM1 globular C1q-homology region are herein designated gAPM1 polypeptide fragments.) APM1 is therefore a ligand of the extracellular domain of TNFRSF2 polypeptide. C2P and D2P are comprised of a globular C1q-homology region that is homologous to that of APM1. APM1, C2P, and D2P globular C1q-homology regions are highly related in sequence at the amino acid level.

Thus, the invention is drawn to FRADJ and/or CRYPTIC polypeptide, polynucleotides encoding said FRADJ and/or CRYPTIC polypeptide, vectors comprising said FRADJ and/or CRYPTIC polynucleotides, and cells recombinant for said FRADJ and/or CRYPTIC polynucleotides, as well as to pharmaceutical and physiologically acceptable compositions comprising said FRADJ and/or CRYPTIC polypeptide fragments and methods of administering said pharmaceutical and physiologically acceptable compositions in order to increase or reduce body weight or to treat obesity-related diseases and disorders. Assays for identifying AGONISTS and ANTAGONISTS of obesity-related activity are also part of the invention.

The present invention provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) the full length sequence of SEQ ID NO:1; (b) a sequence of SEQ ID NO:1 encoding full length FRADJ of SEQ ID NO:2; (c) a sequence of SEQ ID NO:1 encoding full length FRADJ of SEQ ID NO:2 absent the N-terminal Met; (d) a sequence of SEQ ID NO:1 encoding mature FRADJ protein of SEQ ID NO:2 lacking signal peptide; (e) a sequence of SEQ ID NO:1 encoding the extracellular domain of FRADJ protein of SEQ ID NO:2; (f) a sequence of SEQ ID NO:1 encoding the transmembrane domain of FRADJ protein of SEQ ID NO:2; (g) a sequence of SEQ ID NO:1 encoding the intracellular domain of FRADJ protein of SEQ ID NO:2; (h) a sequence of SEQ ID NO:1 encoding the LIGAND binding domain of FRADJ protein of SEQ ID NO:2; (i) a sequence of SEQ ID NO:1 encoding FRADJ polypeptide of protein of SEQ ID NO:2 wherein said FRADJ polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (j) the variant polynucleotides of any of the polynucleotides of (a)-(i); (k) the polynucleotides comprising a nucleotide sequence of (a)-(j), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (l) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (k). The invention further provides for fragments of the nucleic acid molecules of (a)-(l) described above.

The present invention provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) the full length sequence of SEQ ID NO:3; (b) a sequence of SEQ ID NO:1 encoding full length CRYPTIC of SEQ ID NO:4; (c) a sequence of SEQ ID NO:3 encoding full length CRYPTIC of SEQ ID NO:4 absent the N-terminal Met; (d) a sequence of SEQ ID NO:3 encoding mature CRYPTIC protein of SEQ ID NO:4 lacking signal peptide; (e) a sequence of SEQ ID NO:3 encoding the extracellular domain of CRYPTIC protein of SEQ ID NO:4; (f) a sequence of SEQ ID NO:3 encoding the transmembrane domain of CRYPTIC protein of SEQ ID NO:4; (g) a sequence of SEQ ID NO:3 encoding the intracellular domain of CRYPTIC protein of SEQ ID NO:4; (h) a sequence of SEQ ID NO:3 encoding the LIGAND binding domain of CRYPTIC protein of SEQ ID NO:4; (i) a sequence of SEQ ID NO:3 encoding CRYPTIC polypeptide of protein of SEQ ID NO:4 wherein said CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (j) the variant polynucleotides of any of the polynucleotides of (a)-(i); (k) the polynucleotides comprising a nucleotide sequence of (a)-(j), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (l) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (k). The invention further provides for fragments of the nucleic acid molecules of (a)-(l) described above.

Further embodiments of the invention include a purified or isolated polynucleotide that comprise, consist of, or consist essentially of a nucleotide sequence at least 70% identical, more preferably at least 75%, and even more preferably at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical, to any of the nucleotide sequences in (a)-(l) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a)-(l) above.

The present invention additionally provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) a polynucleotide encoding full length FRADJ of SEQ ID NO:2; (b) a polynucleotide encoding full length FRADJ of SEQ ID NO:2 absent the N-terminal Met; (c) a polynucleotide encoding mature FRADJ protein of SEQ ID NO:2; (d) a polynucleotide encoding the extracellular domain of FRADJ protein of SEQ ID NO:2; (e) a polynucleotide encoding the transmembrane domain of FRADJ protein of SEQ ID NO:2; (f) a polynucleotide encoding the intracellular domain of FRADJ protein of SEQ ID NO:2; (g) a polynucleotide encoding the LIGAND binding domain of FRADJ protein of SEQ ID NO:2; (h) a polynucleotide encoding FRADJ polypeptide of protein of SEQ ID NO:2 wherein said FRADJ polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the variant polynucleotides of any of the polynucleotides of (a)-(h); (j) the polynucleotides comprising a nucleotide sequence of (a)-(i), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (k) the polynucleotides

comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (j). The invention further provides for fragments of the nucleic acid molecules of (a)-(k) described above.

The present invention additionally provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) a polynucleotide encoding full length CRYPTIC of SEQ ID NO:4; (b) a polynucleotide encoding full length CRYPTIC of SEQ ID NO:4 absent the N-terminal Met; (c) a polynucleotide encoding mature CRYPTIC protein of SEQ ID NO:4; (d) a polynucleotide encoding the extracellular domain of CRYPTIC protein of SEQ ID NO:4; (e) a polynucleotide encoding the transmembrane domain of CRYPTIC protein of SEQ ID NO:4; (f) a polynucleotide encoding the intracellular domain of CRYPTIC protein of SEQ ID NO:4; (g) a polynucleotide encoding the LIGAND binding domain of CRYPTIC protein of SEQ ID NO:4; (h) a polynucleotide encoding CRYPTIC polypeptide of protein of SEQ ID NO:4 wherein said CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the variant polynucleotides of any of the polynucleotides of (a)-(h); (j) the polynucleotides comprising a nucleotide sequence of (a)-(i), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (k) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (j). The invention further provides for fragments of the nucleic acid molecules of (a)-(k) described above.

Further embodiments of the invention include a purified or isolated polynucleotide that comprise, consist of, or consist essentially of a nucleotide sequence at least 70% identical, more preferably at least 75%, and even more preferably at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical, to any of the nucleotide sequences in (a)-(k) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a)-(k) above.

The present invention also relates to recombinant vectors, which include the purified or isolated polynucleotides of the present invention, and to host cells recombinant for the polynucleotides of the present invention, as well as to methods of making such vectors and host cells. The present invention further relates to the use of these recombinant vectors and recombinant host cells in the production of FRADJ and/or CRYPTIC polypeptides.

The invention further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) the full length FRADJ polypeptide of protein of SEQ ID NO:2; (b) the full length FRADJ polypeptide of protein of SEQ ID NO:2 absent the N-terminal Met; (c) mature FRADJ polypeptide of protein of SEQ ID NO:2 lacking signal peptide; (d) the extracellular domain of FRADJ polypeptide of protein of SEQ ID NO:2; (e) the transmembrane domain of FRADJ polypeptide of protein of SEQ ID NO:2; (f) the intracellular

domain of FRADJ polypeptide of protein of SEQ ID NO:2; (g) a LIGAND binding fragment of FRADJ polypeptide of protein SEQ ID NO:2; (h) FRADJ polypeptide of protein of SEQ ID NO:2 wherein said FRADJ polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the epitope-bearing fragments of FRADJ polypeptide of protein of SEQ ID NO:2; (j) the allelic variant polypeptides of any of the polypeptides of (a)-(i). The invention further provides for fragments of the polypeptides of (a)-(i) above, such as those having biological activity or comprising biologically functional domain(s).

The invention further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consistin g of: (a) the full length CRYPTIC polypeptide of protein of SEQ ID NO:4; (b) the full length CRYPTIC polypeptide of protein of SEQ ID NO:4 absent the N-terminal Met; (c) mature CRYPTIC polypeptide of protein of SEQ ID NO:4 lacking signal peptide; (d) the extracellular domain of CRYPTIC polypeptide of protein of SEQ ID NO:4; (e) the transmembrane domain of CRYPTIC polypeptide of protein of SEQ ID NO:4; (f) the intracellular domain of CRYPTIC polypeptide of protein of SEQ ID NO:4; (g) a LIGAND binding fragment of CRYPTIC polypeptide of protein SEQ ID NO:4; (h) CRYPTIC polypeptide of protein of SEQ ID NO:4 wherein said CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the epitope-bearing fragments of CRYPTIC polypeptide of protein of SEQ ID NO:4; (j) the allelic variant polypeptides of any of the polypeptides of (a)-(i). The invention further provides for fragments of the polypeptides of (a)-(i) above, such as those having biological activity or comprising biologically functional domain(s).

The present invention further includes polypeptides with an amino acid sequence with at least 70% similarity, and more preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to those polypeptides described in (a)-(i), as well as polypeptides having an amino acid sequence at least 70% identical, more preferably at least 75% identical, and still more preferably 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to those polypeptides described in (a)-(i). The invention further relates to methods of making the polypeptides of the present invention.

The present invention further relates to transgenic plants or animals, wherein said transgenic plant or animal is transgenic for a polynucleotide of the present invention and expresses a polypeptide of the present invention.

The invention further relates to antibodies that specifically bind to the FRADJ and/or CRYPTIC polypeptides of the present invention and fragments thereof as well as to methods for producing such antibodies and fragments thereof.

The present invention also provides a method of assaying test molecules to identify a test molecule that binds to FRADJ and/or CRYPTIC polypeptide. The method comprises contacting FRADJ and/or CRYPTIC polypeptide with a test molecule and to determine the extent of binding of the test

molecule to said FRADJ and/or CRYPTIC polypeptide. The method further comprises determining whether such test compounds are AGONISTS or ANTAGONISTS of FRADJ and/or CRYPTIC polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of FRADJ and/or CRYPTIC polypeptide or on the activity of FRADJ and/or CRYPTIC polypeptide.

The invention also provides kits and methods of detecting FRADJ and/or CRYPTIC expression and/or biological activity in a biological sample. One such method involves assaying for the expression of a FRADJ and/or CRYPTIC polynucleotide in a biological sample using polymerase chain reaction (PCR) to amplify and detect FRADJ and/or CRYPTIC polynucleotides or southern and northern blot hybridization to detect FRADJ and/or CRYPTIC genomic DNA, cDNA or mRNA. Alternatively, a method of detecting FRADJ and/or CRYPTIC expression in a test sample can be accomplished using a compound that binds to a FRADJ and/or CRYPTIC polypeptide of the present invention or a portion of a FRADJ and/or CRYPTIC polypeptide.

The present invention also relates to diagnostic methods of identifying individuals or non-human animals having elevated or reduced levels of FRADJ and/or CRYPTIC products, which individuals are likely to benefit from therapies to suppress or enhance FRADJ and/or CRYPTIC expression, respectively, and to methods of identifying individuals or non-human animals at increased risk for developing, or present state of having, certain diseases/disorders associated with FRADJ and/or CRYPTIC abnormal expression or biological activity.

The present invention also relates to kits and methods of screening compounds for their ability to modulate (e.g. increase or inhibit) the activity or expression of FRADJ and/or CRYPTIC including compounds that interact with FRADJ and/or CRYPTIC regulatory sequences and compounds that interact directly or indirectly with FRADJ and/or CRYPTIC polypeptides. Uses of such compounds are also under the scope of the present invention.

The present invention provides for methods of identifying antagonists of FRADJ and/or CRYPTIC polypeptide biological activity comprising contacting a small molecule compound with FRADJ and/or CRYPTIC polypeptides and measuring FRADJ and/or CRYPTIC polypeptide biological activity in the presence and absence of these small molecules. The present invention further provides for methods of identifying agonists of FRADJ and/or CRYPTIC polypeptide biological activity comprising contacting a small molecule compound with FRADJ and/or CRYPTIC polypeptides and measuring FRADJ and/or CRYPTIC polypeptide biological activity in the presence and absence of these small molecules. These small molecules can be a naturally occurring medicinal compound or derived from combinatorial chemical libraries.

The present invention also relates to pharmaceutical or physiologically acceptable compositions comprising, an active agent, the polypeptides, polynucleotides or antibodies of the present invention.

In a first aspect, the invention is directed to a FRADJ and/or CRYPTIC ANTAGONIST, wherein said ANTAGONIST is a soluble fragment of FRADJ and/or CRYPTIC polypeptide, an antibody th at specifically binds FRADJ and/or CRYPTIC, a compound excluding said soluble fragment of FRADJ and/or CRYPTIC polypeptide and said FRADJ and/or CRYPTIC antibody (e.g., small molecular weight organic or inorganic compound, protein, peptide, carbohydrate, l ipid), or a variant or fragment of LIGAND polypeptide.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC ANTAGONIST, wherein said ANTAGONIST is a soluble fragment of FRADJ and/or CRYPTIC polypeptide. More preferably the invention is directed to purified, isolated, or recombinant soluble fragments of FRADJ and/or CRYPTIC polypeptide. More preferably the invention is directed to said soluble fragment of FRADJ and/or CRYPTIC polypeptide, wherein said soluble fragment bind s LIGAND and blocks LIGAND activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said soluble fragment of FRADJ and/or CRYPTIC polypeptide does not activate FRADJ and/or CRYPTIC. Preferably said soluble fragment of FRADJ and/or CRYPTIC polypeptide blocks or inhibits LIGAND binding to FRADJ and/or CRYPTIC. In preferred embodiments, said soluble fragment of FRADJ and/or CRYPTIC polypeptide comprises, consists essentially of, or consists of, at least 6 and not more than 129 consecutive amino acids of SEQ ID NO:2 or 4, more preferably of amino acids comprising the extracellular domain of FRADJ or CRYPTIC, respectively. Preferred said soluble fragment of FRADJ and/or CRYPTIC comprises the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28 -74, or 28-76 or 28-77 of SEQ ID NO:2 or 4, respectivley, where it is understood that amino acid 28 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide. In other preferred embodiments, said soluble fragment of FRADJ and/or CRYPTIC polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of SEQ ID NO:2 or 4, respectively. Further preferred embodiments include heterologous p olypeptides comprising a FRADJ and/or CRYPTIC polypeptide of the invention. In further preferred embodiment, a FRADJ and/or CRYPTIC polypeptide of the invention is conjugated at its N- or C-terminus to an antibody Fc region or portion thereof.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC ANTAGONIST, wherein said ANTAGONIST is an antibody that specifically binds FRADJ and/or CRYPTIC. More preferably the invention is directed to said FRADJ and/or CRYPTIC antibody, w herein said FRADJ and/or CRYPTIC antibody binds FRADJ and/or CRYPTIC and blocks LIGAND activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said FRADJ and/or CRYPTIC antibody does not activate

FRADJ and/or CRYPTIC. Preferably said FRADJ and/or CRYPTIC antibody blocks or inhibits LIGAND binding to FRADJ and/or CRYPTIC.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC ANTAGONIST, wherein said ANTAGONIST is a compound excluding said soluble fragment of FRADJ and/or CRYPTIC polypeptide and said FRADJ and/or CRYPTIC antibody (e.g., small organic molecule, protein, peptide). More preferably the invention is directed to said compound, wherein said compound binds to FRADJ and/or CRYPTIC and blocks LIGAND activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said compound does not activate FRADJ and/or CRYPTIC. Preferably said compound that binds to FRADJ and/or CRYPTIC blocks or inhibits LIGAND binding to FRADJ and/or CRYPTIC. Further more preferably the invention is directed to said compound, wherein said compound blocks or inhibits LIGAND activity exclusive of binding to FRADJ and/or CRYPTIC, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said compound does not activate FRADJ and/or CRYPTIC. Further more preferably the invention is directed to said compound, wherein said compound blocks or inhibits FRADJ and/or CRYPTIC expression and wherein said compound does not have LIGAND activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said compound does not activate FRADJ and/or CRYPTIC.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC ANTAGONIST, wherein said ANTAGONIST is a variant or fragment of LIGAND polypeptide. More preferably the invention is directed to said variant of fragment of LIGAND polypeptide, wherein said variant or fragment of LIGAND polypeptide binds FRADJ and/or CRYPTIC and blocks LIGAND activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein, and wherein said variant or fragment of LIGAND polypeptide does not activate FRADJ and/or CRYPTIC. Preferably said variant or fragment of LIGAND polypeptide blocks or inhibits LIGAND binding to FRADJ and/or CRYPTIC. More preferably the invention is directed to said variant or fragment of LIGAND polypeptide, wherein said variant or fragment of LIGAND polypeptide inhibits the induction, enhancement, or potentiation of said biological activity exclusive of binding to FRADJ and/or CRYPTIC.

Preferred LIGAND is APM1. Preferred LIGAND polypeptide fragment is APM1 polypeptide fragment. Further preferred is said APM1 polypeptide fragment comprised of amino acids 18-244, 34-244, 49-244, 56-244, 59-244, 66-244, 69-244, 78-244, 85-244, 93-244, 101-244, 102-244, 103-244, or 113-244, wherein said numbering of said amino acids within APM1 amino acid sequence is understood to be taken from said APM1 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30. Preferred LIGAND polypeptide fragment is ACRP30 polypeptide fragment. Further preferred is said ACRP30 polypeptide fragment comprised of amino acids 18-247, 37-247, 59-247, 62-247, 69-247, 72-247, 81-247, 88-247, 96-247, 104-247, 105-247, 106-247 or 116-247, wherein said numbering of said amino acids within ACRP30 amino acid sequence is understood to be taken from said ACRP30 amino acid sequence presented in Table 2.

Preferred LIGAND is C2P. Preferred LIGAND polypeptide fragment is C2P polypeptide fragment. Further preferred is said C2P polypeptide fragment comprised of amino acids 20-333, 25-333, 43-333, 45-333, 46-333, 50-333, 53-333, 61-333, 67-333, 74-333, 75-333, 77-333, 81-333, 82-333, 86-333, 89-333, 95-333, 100-333, 104-333, 113-333, 116-333, 125-333, 128-333, 140-333, 160-333, 164-333, 179-333, 182-333, 185-333, 188-333, 191-333, 193-333, or 202-333, wherein said numbering of said amino acids within C2P amino acid sequence is understood to be taken from said C2P amino acid sequence presented in Table 2.

Preferred LIGAND is D2P. Preferred LIGAND polypeptide fragment is D2P polypeptide fragment. Further preferred is said D2P polypeptide fragment comprised of amino acids 20-333, 25-333, 43-333, 45-333, 46-333, 50-333, 53-333, 67-333, 74-333, 75-333, 77-333, 81-333, 82-333, 86-333, 89-333, 95-333, 100-333, 104-333, 113-333, 116-333, 125-333, 128-333, 140-333, 160-333, 164-333, 179-333, 182-333, 185-333, 188-333, 191-333, 193-333, or 202-333, wherein said numbering of said amino acids within D2P amino acid sequence is understood to be taken from said D2P amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R1. Preferred LIGAND polypeptide fragment is ACRP30R1 polypeptide fragment. Further preferred is said ACRP30R1 polypeptide fragment comprised of amino acids 16-217, 25-217, 26-217, 29-217, 30-217, 47-217, 60-217, 66-217, 76-217, or 87-217, wherein said numbering of said amino acids within ACRP30R1 amino acid sequence is understood to be taken from said ACRP30R1 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R1L. Preferred LIGAND polypeptide fragment is ACRP30R1L polypeptide fragment. Further preferred is said ACRP30R1L polypeptide fragment comprised of amino acids 16-285, 25-285, 26-285, 29-285, 30-285, 91-285, 93-285, 97-285, 98-285, 99-285, 105-285, 109-285, 112-285, 120-285, 126-285, 127-285, 130-285, 132-285, 133-285, 134-285, or 150-285, wherein said numbering of said amino acids within ACRP30R1L amino acid sequence is understood to be taken from said ACRP30R1L amino acid sequence presented in Table 2.

Preferred LIGAND is HGS. Preferred LIGAND polypeptide fragment is HGS polypeptide fragment. Further preferred is said HGS polypeptide fragment comprised of amino acids 57-288, 90-288, 98-288, 107-288, 110-288, 124-288, 137-288, 140-288, 144-288, 152-288, or 153-288, wherein said numbering of said amino acids within HGS amino acid sequence is understood to be taken from said HGS amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP6. Preferred LIGAND polypeptide fragment is ZACRP6 polypeptide fragment. Further preferred is said ZACRP6 polypeptide fragment comprised of amino acids 47-278, 80-278, 88-278, 97-278, 100-278, 114-278, 127-278, 130-278, 134-278, 142-278, or 143-278, wherein said numbering of said amino acids within ZACRP6 amino acid sequence is understood to be taken from said ZACRP6 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R2. Preferred LIGAND polypeptide fragment is ACRP30R2 polypeptide fragment. Further preferred is said ACRP30R2 polypeptide fragment comprised of amino acids 28-259, 61-259, 69-259, 78-259, 81-259, 95-259, 108-259, 111-259, 115-259, 123-259, or 124-259, wherein said numbering of said amino acids within ACRP30R2 amino acid sequence is understood to be taken from said ACRP30R2 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP5. Preferred LIGAND polypeptide fragment is ZACRP5 polypeptide fragment. Further preferred is said ZACRP5 polypeptide fragment comprised of amino acids 18-252, 24-252, 25-252, 32-252, 42-252, 46-252, 50-252, 55-252, 59-252, 61-252, 70-252, 73-252, 79-252, 81-252, 84-252, 87-252, 89-252, 94-252, 99-252, 100-252, 103-252, 107-252, 114-252, or 115-252, wherein said numbering of said amino acids within ZACRP5 amino acid sequence is understood to be taken from said ZACRP5 amino acid sequence presented in Table 2.

Preferred LIGAND is ZSIG37. Preferred LIGAND polypeptide fragment is ZSIG37 polypeptide fragment. Further preferred is said ZSIG37 polypeptide fragment comprised of amino acids 26-281, 60-281, 62-281, 73-281, 76-281, 99-281, 102-281, 105-281, 108-281, 113-281, 116-281, 118-281, 123-281, 129-281, 132-281, 133-281, 141-281, or 143-281, wherein said numbering of said amino acids within ZSIG37 amino acid sequence is understood to be taken from said ZSIG37 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP4. Preferred LIGAND polypeptide fragment is ZACRP4 polypeptide fragment. Further preferred is said ZACRP4 polypeptide fragment comprised of amino acids 17-329, 17-167, 17-176, 30-329, 30-167, 30-176, 168-329, or 177-329, wherein said numbering of said amino acids within ZACRP4 amino acid sequence is understood to be taken from said ZACRP4 amino acid sequence presented in Table 2.

Preferred LIGAND is ZSIG39. Preferred LIGAND polypeptide fragment is ZSIG39 polypeptide fragment. Further preferred is said ZSIG39 polypeptide fragment comprised of amino acids 16-243, 24-243, 50-243, 53-243, 56-243, 58-243, 66-243, 71-243, 78-243, 84-243, or 104-243, wherein said numbering of said amino acids within ZSIG39 amino acid sequence is understood to be taken from said ZSIG39 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP3. Preferred LIGAND polypeptide fragment is ZACRP3 polypeptide fragment. Further preferred is said ZACRP3 polypeptide fragment comprised of amino acids 23-246, 42-246, 51-246, 84-246, 87-246, 90-246, 96-246, 99-246, 105-246, or 108-246, wherein said

numbering of said amino acids within ZACRP3 amino acid sequence is understood to be taken from said ZACRP3 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP7. Preferred LIGAND polypeptide fragment is ZACRP7 polypeptide fragment. Further preferred is said ZACRP7 polypeptide fragment comprised of amino acids 31-303, 39-303, 78-303, 81-303, 84-303, 85-303, 88-303, 91-303, 97-303, 99-303, 109-303, 117-303, 118-303, 127-303, 139-303, 142-303, 155-303, or 162-303, wherein said numbering of said amino acids within ZACRP7 amino acid sequence is understood to be taken from said ZACRP3 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-3. Preferred LIGAND polypeptide fragment is C1RF-3 polypeptide fragment. Further preferred is C1RF-3 polypeptide fragment comprised of amino acids 22-287, 31-287, 49-287, 79-287, 84-287, 87-287, 91-287, 103-287, 109-287, 114-287, 151-287, or 159-287, wherein said numbering of said amino acids within C1RF-3 amino acid sequence is understood to be taken from said C1RF-3 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-2. Preferred LIGAND polypeptide fragment is C1RF-2 polypeptide fragment. Further preferred is C1RF-2 polypeptide fragment comprised of amino acids 21-255, 30-255, 40-255, 50-255, 64-255, 69-255, 72-255, 76-255, 94-255, 104-255, 117-255, or 127-255, wherein said numbering of said amino acids within C1RF-2 amino acid sequence is understood to be taken from said C1RF-2 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-1. Preferred LIGAND polypeptide fragment is C1RF-1 polypeptide fragment. Further preferred is C1RF-1 polypeptide fragment comprised of amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 100-258, 101-258, 105-258, 115-258, or 130-258, wherein said numbering of said amino acids within C1RF-1 amino acid sequence is understood to be taken from said C1RF-1 amino acid sequence presented in Table 2.

Most preferred LIGAND is APM1, C2P, or D2P. Particularly most preferred LIGAND is APM1. Most preferred LIGAND polypeptide fragment is said APM1 polypeptide fragment, said C2P polypeptide fragment, or said D2P polypeptide fragment. Particularly most preferred LIGAND polypeptide fragment is said APM1 polypeptide fragment.

In a further preferred embodiment, said ANTAGONIST is able to raise circulating (either blood, serum or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides.

Further preferred said ANTAGONISTS are those that significantly inhibit muscle lipid or free fatty acid oxidation stimulated by its LIGAND. Further preferred said ANTAGONISTS are those that cause C2C12 cells differentiated in the presence of LIGAND to undergo at least 10%, 20%, 30%, 35%, or 40% less oleate oxidation as compared to untreated cells.

Further preferred said ANTAGONISTS are those that inhibit by at least 10%, 20%, 30%, 35%, or 40% the increase in leptin uptake stimulated by LIGAND polypeptide in a liver cell line [preferably BPRCL mouse liver cells (ATCC CRL-2217)] as compared to untreated cells.

Further preferred said ANTAGONISTS are those that significantly increase the postprandial increase in plasma free fatty acids, particularly following a high fat meal.

Further preferred said ANTAGONISTS are those that significantly increase ketone body production, particularly following a high fat meal.

Further preferred said ANTAGONISTS are those that decrease glucose uptake in skeletal muscle cells stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that decrease glucose uptake in adipose cells stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that decrease glucose uptake in neuronal cells stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that decrease glucose uptake in red blood cells stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that decrease glucose uptake in the brain stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that significantly increase the postprandial increase in plasma glucose following a meal, particularly a high carbohydrate meal.

Further preferred said ANTAGONISTS are those that significantly facilitate the postprandial increase in plasma glucose following a meal, particularly a high fat or a high carbohydrate meal.

Further preferred said ANTAGONISTS are those that reduce the insulin sensitivity stimulated by LIGAND.

Further preferred said ANTAGONISTS are those that increase body mass, wherein said increase in body mass is comprised of a change in mass of the subcutaneous adipose tissue.

Further preferred said ANTAGONISTS are those that increase body mass, wherein said increase in body mass is comprised of a change in mass of the visceral (omental) adipose tissue.

Further preferred said ANTAGONISTS are those that form multimers (e.g., heteromultimers or homomultimers) *in vitro* and/or *in vivo*. Preferred multimers are homodimers or homotrimers.

In a second aspect, the invention features a purified, isolated, or recombinant polynucleotide encoding said FRADJ and/or CRYPTIC polypeptide described above, or the complement thereof. In

further embodiments the polynucleotides are DNA, RNA, DNA/RNA hybrids, single-stranded, and double-stranded.

In a third aspect, the invention features a recombinant vector comprising, consisting essentially of, or consisting of, said polynucleotide described in the second aspect.

In a fourth aspect, the invention features a recombinant cell comprising, consisting essentially of, or consisting of, said recombinant vector described in the third aspect. A further embodiment includes a host cell recombinant for a polynucleotide of the invention.

In a fifth aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of, said ANTAGONIST described above and, alternatively, a pharmaceutical or physiologically acceptable diluent.

In a sixth aspect, the invention features a method of increasing body mass comprising providing or administering to individuals in need of increasing body mass said pharmaceutical or physiologically acceptable composition described in the fifth aspect.

In preferred embodiments, the identification of said individuals in need of increasing body mass to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of plasma, urine, and saliva. Preferably, ANTAGONIST of the present invention is administered to an individual with at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% increase in blood, serum or plasma levels of LIGAND polypeptide as compared to healthy patients.

In a seventh aspect, the invention features a method of preventing or treating disorders associated with excessive weight loss comprising providing or administering to an individual in need of such treatment said pharmaceutical or physiologically acceptable composition described in the fifth aspect. In preferred embodiments, the identification of said individuals in need of such treatment to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said LIGAND is APM1, C2P, or D2P. Most preferably, said LIGAND is APM1. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva. Preferably said disorder is selected from the group consisting of cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia. Said disorders associated with excessive weight loss are comprised of those mediated by tumor necrosis factor (TNFalpha) alone, those mediated by TNFalpha plus one or more additional factors, and those mediated only by one or more factors exclusive of TNFalpha. Said factors include, but are not restricted to, macrophage migration inhibitory factor,

interleukin 1, and interleukin 6. In preferred embodiments, said individual is a mammal, preferably a human.

In related aspects, embodiments of the present invention includes methods of causing or inducing a desired biological response in an individual comprising the steps of: pro viding or administering to an individual a composition comprising ANTAGONIST, wherein said biological response is selected from the group consisting of:

(a) raising circulating (either blood, serum, or plasma) levels (concentration) of free fatty acids (FFA) or triglycerides (TG);

(b) raising circulating (either blood, serum or plasma) levels (concentration) of glucose;

(c) raising circulating (either blood, serum or plasma) levels (concentration) of triglycerides;

(d) inhibiting muscle lipid or free fatty acid oxidation;

(c) inhibiting leptin uptake in the liver or liver cells;

(e) increasing the postprandial increase in plasma free fatty acids, particularly following a high fat meal; and,

(f) increasing or eliminating ketone body production, particul arly following a high fat meal;

(g) reducing tissue sensitivity to insulin, particularly muscle, adipose, liver or brain,

and further wherein said biological response is greater than a transient response; or alternatively wherein said biological response is sustained. In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method of increasing body mass in some persons with cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an insulin de-sensitiser, wherein the sensitivity of a cell or tissue to insulin is reduced.

In an eighth aspect, the invention features a method of making the FRADJ and/or CRYPTIC polypeptide described in the first aspect, wherein said method is s elected from the group consisting of: proteolytic cleavage, recombinant methodology and artificial synthesis.

In a ninth aspect, the invention features a use of ANTAGONIST described in the first aspect for the preparation of a medicament for the treatment of disorders associated with excessive weight loss and/or for increasing body mass. Preferably, said disorder is selected from the group consisting of cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-

related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

In an tenth aspect, the invention provides ANTAGONIST of the first aspect of the invention, or a composition of the fifth aspect of the invention, for use in a method of treatment of the human or animal body.

In a eleventh aspect, the invention features methods of increasing body weight comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the fifth aspect, or ANTAGONIST described in the first aspect. Where the increase of body weight is practiced for cosmetic purposes, the individual has a BMI of no greater than 25 and at least 20. In embodiments for the treatment of disorders associated with excessive weight loss, the individual may have a BMI no greater than 20. One embodiment for the treatment of disorders associated with excessive weight loss provides for the treatment of individuals with BMI values of no greater than 15. Alternatively, for increasing the body weight of an individual, the BMI value should be at least 15 and no more than 20.

In a twelfth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect for increasing body mass and/or for treatment of disorders associated with excessive weight loss. Preferably, said disorder is selected from the group consisting of cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human. In preferred embodiments, the identification of said individuals to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva.

In a thirteenth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect for increasing body weight for cosmetic reasons.

In a preferred aspect of the methods above and disclosed herein, the amount of ANTAGONIST administered to an individual is sufficient to bring levels of FRADJ and/or CRYPTIC activition to their normal levels (levels in healthy individuals). "Normal levels" of FRADJ and/or CRYPTIC activation may be followed using surrogate markers including circulating (either blood, serum or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides.

In a fifteenth aspect, the invention is directed to FRADJ and/or CRYPTIC AGONISTS, wherein said AGONIST is an antibody that specifically binds FRADJ and/or CRYPTIC, a compound excluding said FRADJ and/or CRYPTIC antibody (e.g., small organic or inorganic compound, protein, peptide, carbohydrate, lipid), or a LIGAND polypeptide or fragment thereof.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC AGONIST, wherein said AGONIST is an antibody that specifically binds FRADJ and/or CRYPTIC. More preferably the invention is directed to said FRADJ and/or CRYPTIC antibody, wherein said FRADJ and/or CRYPTIC antibody binds FRADJ and/or CRYPTIC and manifests LIGAND activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC AGONIST, wherein said AGONIST is a compound excluding said FRADJ and/or CRYPTIC antibody. More preferably the invention is directed to said compound, wherein said compound binds FRADJ and/or CRYPTIC and manifests LIGAND activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein. Further more preferably the invention is directed to said compound, wherein said compound manifests LIGAND activity exclusive of binding to FRADJ and/or CRYPTIC, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein. Further more preferably the invention is directed to said compound, wherein said compound increases FRADJ and/or CRYPTIC expression.

In a further preferred embodiment, the invention is directed to a FRADJ and/or CRYPTIC AGONIST, wherein said AGONIST is a LIGAND polypeptide or fragment thereof. More preferably the invention is directed to said LIGAND polypeptide or fragment thereof, wherein said LIGAND polypeptide or fragment thereof binds FRADJ and/or CRYPTIC and elicits biological activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein. More preferably the invention is directed to said LIGAND polypeptide or fragment thereof, wherein said LIGAND polypeptide or fragment thereof induces, enhances, or potentiates said biological activity exclusive of binding to FRADJ and/or CRYPTIC.

Preferred LIGAND is APM1. Preferred LIGAND polypeptide fragment is APM1 polypeptide fragment. Further preferred is said APM1 polypeptide fragment comprised of amino acids 18-244, 34-244, 49-244, 56-244, 59-244, 66-244, 69-244, 78-244, 85-244, 93-244, 101-244, 102-244, 103-244, or 113-244, wherein said numbering of said amino acids within APM1 amino acid sequence is understood to be taken from said APM1 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30. Preferred LIGAND polypeptide fragment is ACRP30 polypeptide fragment. Further preferred is said ACRP30 polypeptide fragment comprised of amino acids 18-247, 37-247, 59-247, 62-247, 69-247, 72-247, 81-247, 88-247, 96-247, 104-247, 105-247, 106-247 or 116-247, wherein said numbering of said amino acids within ACRP30 amino acid sequence is understood to be taken from said ACRP30 amino acid sequence presented in Table 2.

Preferred LIGAND is C2P. Preferred LIGAND polypeptide fragment is C2P polypeptide fragment. Further preferred is said C2P polypeptide fragment comprised of amino acids 20 -333, 25-333, 43-333, 45-333, 46-333, 50-333, 53-333, 61-333, 67-333, 74-333, 75-333, 77-333, 81-333, 82-333, 86-333, 89-333, 95-333, 100-333, 104-333, 113-333, 116-333, 125-333, 128-333, 140-333, 160-333, 164-333, 179-333, 182-333, 185-333, 188-333, 191-333, 193-333, or 202-333, wherein said numbering of said amino acids within C2P amino acid sequence is understood to be taken from said C2P amino acid sequence presented in Table 2.

Preferred LIGAND is D2P. Preferred LIGAND polypeptide fragment is D2P polypeptide fragment. Further preferred is said D2P polypeptide fragment comprised of amino acids 20 -333, 25-333, 43-333, 45-333, 46-333, 50-333, 53-333, 67-333, 74-333, 75-333, 77-333, 81-333, 82-333, 86-333, 89-333, 95-333, 100-333, 104-333, 113-333, 116-333, 125-333, 128-333, 140-333, 160-333, 164-333, 179-333, 182-333, 185-333, 188-333, 191-333, 193-333, or 202-333, wherein said numbering of said amino acids within D2P amino acid sequence is understood to be taken from said D2P amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R1. Preferred LIGAND polypeptide fragment is ACRP30R1 polypeptide fragment. Further preferred is said ACRP30R1 polypeptide fragment comprised of amino acids 16-217, 25-217, 26-217, 29-217, 30-217, 47-217, 60-217, 66-217, 76-217, or 87-217, wherein said numbering of said amino acids within ACRP30R1 amino acid sequence is understood to be taken from said ACRP30R1 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R1L. Preferred LIGAND polypeptide fragment is ACRP30R1L polypeptide fragment. Further preferred is said ACRP30R1L polypeptide fragment comprised of amino acids 16-285, 25-285, 26-285, 29-285, 30-285, 91-285, 93-285, 97-285, 98-285, 99-285, 105-285, 109-285, 112-285, 120-285, 126-285, 127-285, 130-285, 132-285, 133-285, 134-285, or 150-285, wherein said numbering of said amino acids within ACRP30R1L amino acid sequence is understood to be taken from said ACRP30R1L amino acid sequence presented in Table 2.

Preferred LIGAND is HGS. Preferred LIGAND polypeptide fragment is HGS polypeptide fragment. Further preferred is said HGS polypeptide fragment comprised of amino acids 57 -288, 90-288, 98-288, 107-288, 110-288, 124-288, 137-288, 140-288, 144-288, 152-288, or 153-288, wherein said numbering of said amino acids within HGS amino acid sequence is understood to be taken from said HGS amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP6. Preferred LIGAND polypeptide fragment is ZACRP6 polypeptide fragment. Further preferred is said ZACRP6 polypeptide fragment comprised of amino acids 47-278, 80-278, 88-278, 97-278, 100-278, 114-278, 127-278, 130-278, 134-278, 142-278, or 143-278, wherein said numbering of said amino acids within ZACRP6 amino acid sequence is understood to be taken from said ZACRP6 amino acid sequence presented in Table 2.

Preferred LIGAND is ACRP30R2. Preferred LIGAND polypeptide fragment is ACRP30R2 polypeptide fragment. Further preferred is said ACRP30R2 polypeptide fragment comprised of amino acids 28-259, 61-259, 69-259, 78-259, 81-259, 95-259, 108-259, 111-259, 115-259, 123-259, or 124-259, wherein said numbering of said amino acids within ACRP30R2 amino acid sequence is understood to be taken from said ACRP30R2 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP5. Preferred LIGAND polypeptide fragment is ZACRP5 polypeptide fragment. Further preferred is said ZACRP5 polypeptide fragment comprised of amino acids 18-252, 24-252, 25-252, 32-252, 42-252, 46-252, 50-252, 55-252, 59-252, 61-252, 70-252, 73-252, 79-252, 81-252, 84-252, 87-252, 89-252, 94-252, 99-252, 100-252, 103-252, 107-252, 114-252, or 115-252, wherein said numbering of said amino acids within ZACRP5 amino acid sequence is understood to be taken from said ZACRP5 amino acid sequence presented in Table 2.

Preferred LIGAND is ZSIG37. Preferred LIGAND polypeptide fragment is ZSIG37 polypeptide fragment. Further preferred is said ZSIG37 polypeptide fragment comprised of amino acids 26 -281, 60-281, 62-281, 73-281, 76-281, 99-281, 102-281, 105-281, 108-281, 113-281, 116-281, 118-281, 123-281, 129-281, 132-281, 133-281, 141-281, or 143-281, wherein said numbering of said amino acids within ZSIG37 amino acid sequence is understood to be taken from said ZSIG37 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP4. Preferred LIGAND polypeptide fragment is ZACRP4 polypeptide fragment. Further preferred is said ZACRP4 polypeptide fragment comprised of amino acids 17-329, 17-167, 17-176, 30-329, 30-167, 30-176, 168-329, or 177-329, wherein said numbering of said amino acids within ZACRP4 amino acid sequence is understood to be taken from said ZACRP4 amino acid sequence presented in Table 2.

Preferred LIGAND is ZSIG39. Preferred LIGAND polypeptide fragment is ZSIG39 polypeptide fragment. Further preferred is said ZSIG39 polypeptide fragment comprised of amino acids 16-243, 24-243, 50-243, 53-243, 56-243, 58-243, 66-243, 71-243, 78-243, 84-243, or 104-243, wherein said numbering of said amino acids within ZSIG39 amino acid sequence is understood to be taken from said ZSIG39 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP3. Preferred LIGAND polypeptide fragment is ZACRP3 polypeptide fragment. Further preferred is said ZACRP3 polypeptide fragment comprised of amino acids 23-246, 42-246, 51-246, 84-246, 87-246, 90-246, 96-246, 99-246, 105-246, or 108-246, wherein said numbering of said amino acids within ZACRP3 amino acid sequence is understood to be taken from said ZACRP3 amino acid sequence presented in Table 2.

Preferred LIGAND is ZACRP7. Preferred LIGAND polypeptide fragment is ZACRP7 polypeptide fragment. Further preferred is said ZACRP7 polypeptide fragment comprised of amino acids 31-303, 39-303, 78-303, 81-303, 84-303, 85-303, 88-303, 91-303, 97-303, 99-303, 109-303, 117-303,

118-303, 127-303, 139-303, 142-303, 155-303, or 162-303, wherein said numbering of said amino acids within ZACRP7 amino acid sequence is understood to be taken from said ZACRP3 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-3. Preferred LIGAND polypeptide fragment is C1RF-3 polypeptide fragment. Further preferred is C1RF-3 polypeptide fragment comprised of amino acids 22-287, 31-287, 49-287, 79-287, 84-287, 87-287, 91-287, 103-287, 109-287, 114-287, 151-287, or 159-287, wherein said numbering of said amino acids within C1RF-3 amino acid sequence is understood to be taken from said C1RF-3 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-2. Preferred LIGAND polypeptide fragment is C1RF-2 polypeptide fragment. Further preferred is C1RF-2 polypeptide fragment comprised of amino acids 21-255, 30-255, 40-255, 50-255, 64-255, 69-255, 72-255, 76-255, 94-255, 104-255, 117-255, or 127-255, wherein said numbering of said amino acids within C1RF-2 amino acid sequence is understood to be taken from s aid C1RF-2 amino acid sequence presented in Table 2.

Preferred LIGAND is C1RF-1. Preferred LIGAND polypeptide fragment is C1RF-1 polypeptide fragment. Further preferred is C1RF-1 polypeptide fragment comprised of amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 100-258, 101-258, 105-258, 115-258, or 130-258, wherein said numbering of said amino acids within C1RF-1 amino acid sequence is understood to be taken from said C1RF-1 amino acid sequence presented in Table 2.

Most preferred LIGAND is APM1, C2P, or D2P. Particularly most preferred LIGAND is APM1. Most preferred LIGAND polypeptide fragment is said APM1 polypeptide fragment, said C2P polypeptide fragment, or said D2P polypeptide fragment. Particularly most preferred LIGAND polypeptide fragment is said APM1 polypeptide fragment.

In a further preferred embodiment, said AGONIST is able to lower circulating (either blood, serum or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides.

Further preferred AGONISTS are those that significantly stimulate muscle lipid or free fatty acid oxidation as compared to untreated cells. Further preferred AGONISTS are those that cause C2C12 cells differentiated in the presence of said AGONISTS to undergo at least 10%, 20%, 30%, 35%, or 40% more oleate oxidation as compared to untreated cells.

Further preferred AGONISTS are those that increase by at least 10%, 20%, 30%, 35%, or 40% leptin uptake in a liver cell line [preferably BPRCL mouse liver cells (ATCC CRL-2217)] as compared to untreated cells.

Further preferred AGONISTS are those that significantly reduce the postprandial increase in plasma free fatty acids or triglycerides, particularly following a high fat meal.

Further preferred AGONISTS are those that significantly reduce or eliminate ketone body production, particularly following a high fat meal.

Further preferred AGONISTS are those that increase glucose uptake in skeletal muscle cells.

Further preferred AGONISTS are those that increase glucose uptake in adipose cells.

Further preferred AGONISTS are those that increase glucose uptake in neuronal cells.

Further preferred AGONISTS are those that increase glucose uptake in red blood cells.

Further preferred AGONISTS are those that increase glucose uptake in the brain.

Further preferred AGONISTS are those that significantly reduce the postprandial increase in plasma glucose following a meal, particularly a high carbohydrate meal.

Further preferred AGONISTS are those that significantly prevent the postprandial increase in plasma glucose following a meal, particularly a high fat or a high carbohydrate meal.

Further preferred AGONISTS are those that improve insulin sensitivity.

Further preferred said AGONISTS are those that decrease body mass, wherein said decrease in body mass is comprised of a change in mass of the subcutaneous adipose tissue.

Further preferred said AGONISTS are those that decrease body mass, wherein said decrease in body mass is comprised of a change in mass of the visceral (omental) adipose tissue.

In a sixteenth aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of, said AGONIST described in the fifteenth aspect and, alternatively, a pharmaceutical or physiologically acceptable diluent.

In an seventeenth aspect, the invention features a method of reducing body mass comprising providing or administering to individuals in need of reducing body mass said pharmaceutical or physiologically acceptable composition described in the sixteenth aspect.

In preferred embodiments, the identification of said individuals in need of reducing body mass to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said LIGAND is APM1, C2P, or D2P. Most preferably, said LIGAND is APM1. Preferably, said clinical samples are selected from the group consisting of plasma, urine, and saliva. Preferably, AGONIST of the present invention is administered to an individual with at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in blood, serum or plasma levels of LIGAND polypeptide as compared to healthy patients without obesity-related disease or disorder.

In an eighteenth aspect, the invention features a method of preventing or treating an obesity-related disease or disorder comprising providing or administering to an individual in need of such treatment said pharmaceutical or physiologically acceptable composition described in the sixteenth aspect. In preferred embodiments, the identification of said individuals in need of such treatment to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said LIGAND is APM1, C2P, or D2P. Most preferably, said LIGAND is APM1. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva. Preferably, said obesity-related disease or disorder is selected from the group consisting of obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by said FRADJ and/or CRYPTIC AGONIST of the invention include hyperlipidemia and hyperuricemia. In preferred embodiments, said individual is a mammal, preferably a human.

In related aspects, embodiments of the present invention includes methods of causing or inducing a desired biological response in an individual comprising the steps of: providing or administering to an individual a composition comprising AGONIST, wherein said biological response is selected from the group consisting of:

(a) lowering circulating (either blood, serum, or plasma) levels (concentration) of free fatty acids;

(b) lowering circulating (either blood, serum or plasma) levels (concentration) of glucose;

(c) lowering circulating (either blood, serum or plasma) levels (concentration) of triglycerides;

(d) stimulating muscle lipid or free fatty acid oxidation;

(c) increasing leptin uptake in the liver or liver cells;

(e) reducing the postprandial increase in plasma free fatty acids, particularly following a high fat meal;

(f) reducing or eliminating ketone body production, particularly following a high fat meal;

(g) increasing tissue sensitivity to insulin, particularly muscle, adipose, liver or brain,

and further wherein said biological response is significantly greater than, or at least 10%, 20%, 30% , 35%, or 40% greater than that observed in the absence of treatment; or alternatively wherein said biological response is greater than a transient response; or alternatively wherein said biological response is sustained. In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) alone, without combination of insulin therapy.

In a further preferred embodiment, the present invention may be used in complementary therapy of NIDDM patients to improve their weight or glucose control in combination with an insulin secretagogue or an insulin sensitising agent. Preferably, the insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl)guanidine fumarate (BTS67582) or a sulphonylurea selected from tolbutamide,

tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

The present invention further provides a method of improving the body weight or glucose control of NIDDM patients alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, the present invention may be used in complementary therapy of IDDM patients to improve their weight or glucose control in combination with an insulin secretagogue or an insulin sensitising agent. Preferably, the insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl) guanidine fumarate (BTS67582) or a sulphonylurea selected from tolbutamide, tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

The present invention further provides a method of improving the body weight or glucose control of IDDM patients alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, the present invention may be administered either concomitantly or concurrently, with the insulin secretagogue or insulin sensitising agent for example in the form of separate dosage units to be used simultaneously, separately or sequentially (either before or after the secretagogue or either before or after the sensitising agent). Accordingly, the present invention further provides for a composition of pharmaceutical or physiologically acceptable composition and an insulin secretagogue or insulin sensitising agent as a combined preparation for simultaneous, separate or sequential use for the improvement of body weight or glucose control in NIDDM or IDDM patients.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an insulin sensitiser.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Noninsulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) without insulin therapy.

In a nineteenth aspect, the invention features a use of AGONIST described in the fifteenth aspect for treatment of obesity-related diseases and disorders and/or reducing body mass. Preferably, said obesity-related diseases and disorders are selected from the group consisting of obesity, insulin

resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by said AGONIST of the invention include hyperlipidemia and hyperuricemia.

In a twentieth aspect, the invention features a use of AGONIST described in the sixteenth aspect for the preparation of a medicament for the treatment of obesity-related diseases and disorders and/or for reducing body mass. Preferably, said obesity-related disease or disorder is selected from the group consisting of obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. In preferred embodiments, said individual is a mammal, preferably a human.

In a twenty-first aspect, the invention provides AGONIST of the fifteenth aspect of the invention, or a composition of the sixteenth aspect of the invention, for use in a method of treatment of the human or animal body.

In a twenty-second aspect, the invention features methods of reducing body weight comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the sixteenth aspect, or AGONIST described in the fifteenth aspect. Where the reduction of body weight is practiced for cosmetic purposes, the individual has a BMI of at least 20 and no more than 25. In embodiments for the treatment of obesity, the individual may have a BMI of at least 20. One embodiment for the treatment of obesity provides for the treatment of individuals with BMI values of at least 25. Another embodiment for the treatment of obesity provides for the treatment of individuals with BMI values of at least 30. Yet another embodiment provides for the treatment of individuals with BMI values of at least 40.

In a twenty-third aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the sixteenth aspect for reducing body mass and/or for treatment or prevention of obesity-related diseases or disorders. Preferably, said obesity-related disease or disorder is selected from the group consisting of obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type

II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. In preferred embodiments, said individual is a mammal, preferably a human. In preferred embodiments, the identification of said individuals to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping LIGAND single nucleotide polymorphisms (SNPs) or measuring LIGAND polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva.

In a twenty-fourth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the sixteenth aspect for reducing body weight for cosmetic reasons.

In a twenty-fifth aspect, AGONIST of the invention is used in methods of treating insulin resistance comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the sixteenth aspect, or AGONIST described in the fifteenth aspect.

In a preferred aspect of the methods above and disclosed herein, the amount of AGONIST administered to an individual is sufficient to bring levels of FRADJ and/or CRYPTIC activition to their normal levels (levels in individuals without obesity-related disease or disorder). "Normal levels" of FRADJ and/or CRYPTIC activation may be followed using surrogate markers including circulating (either blood, serum or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides.

Brief Description of Tables

Table 1.1 lists known or predicted biologic structural and functional domains for the FRADJ polypeptide of SEQ ID NO:2 of the present invention, including the signal peptide, extracellular (EC) domain, transmembrane domain, and intracellular (IC) domain.

Table 1.2 lists known or predicted biologic structural and functional domains for the CRYPTIC polypeptide of SEQ ID NO:4 of the present invention, including the signal peptide, extracellular (EC) domain, transmembrane domain, and intracellular (IC) domain.

Table 2 lists the LIGANDS of the present invention. Presented are polypeptide amino acid sequences. C1q homology globular domain homologous to APM1 C1q homology globular domain is indicated by underlining. The putative signal peptide is indicated in bold. The number of amino acids comprising the polypeptide sequence is indicated in parentheses. APM1, C2P, and D2P are preferred LIGANDS of the invention. APM1 is most preferred LIGAND of the invention.

Brief Description of Sequence Listing

SEQ ID NO:1 is the nucleotide sequence of FRADJ cDNA with an open reading frame which location is indicated as features. When appropriate, the locations of the potential polyadenylation site and polyadenylation signal are also indicated.

SEQ ID NO:2 is the amino acid sequence of protein encoded by the cDNA of SEQ ID NO:1.

SEQ ID NO:3 is the nucleotide sequence of CRYPTIC cDNA with an open reading frame which location is indicated as features. When appropriate, the locations of the potential polyadenylation site and polyadenylation signal are also indicated.

SEQ ID NO:4 is the amino acid sequence of protein encoded by the cDNA of SEQ ID NO:3.

The appended Sequence Listing is hereby incorporated by reference in it s entirety.

Detailed Description

Definitions

Before describing the invention in greater detail, the following definitions are set forth to illustrate and define the meaning and scope of the terms used to describe the invention herein.

The terms "FRADJ and/or CRYPTIC gene", when used herein, encompasses genomic, mRNA and cDNA sequences encoding the FRADJ and/or CRYPTIC protein, including the 5' and 3' untranslated regions of said sequences.

The term "FRADJ and/or CRYPTIC biological activity" is intended for polypeptides exhibiting similar, but not necessarily identical, to an activity of the FRADJ and/or CRYPTIC polypeptide of the invention, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or described herein. The FRADJ and/or CRYPTIC biological activity of a given polypeptide may be assessed using a suitable biological assay well known to those skilled in the art [such as one described in the Examples herein]. In contrast, th e term "biological activity" refers to any activity that a polypeptide of the invention may have.

The term "corresponding mRNA" refers to the mRNA that was the template for the cDNA synthesis that produced a cDNA of the present invention.

The term "corresponding genomic DNA" refers to the genomic DNA which encodes mRNA which includes the sequence of one of the strands of the cDNA in which thymidine residues in the sequence of the cDNA are replaced by uracil residues in the mRNA.

The term "heterologous", when used herein, is intended to designate any polynucleotide or polypeptide other than the FRADJ and/or CRYPTIC polynucleotide or polypeptide respectively.

The term "isolated" requires that the material be removed from its original environment (e. g., the natural environment if the material is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment. For example, a naturally occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated. Specifically excluded from the definition of "isolated" are: naturally-occurring chromosomes (such as chromosome spreads), artificial chromosome libraries, genomic libraries, and cDNA libraries that exist either as an *in vitro* nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an *in vitro* heterogeneous preparation or plated as a heterogeneous population of single colonies. Also specifically excluded are the above libraries wherein a specified polynucleotide makes up less than 5% of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including said whole cell preparations which are mechanically sheared or enzymatically digested). Further specifically excluded are the above whole cell preparations as either an *in vitro* preparation or as a heterogeneous mixture separated by electrophoresis (including blot transfers of the same) wherein the polynucleotide of the invention has not further been separated from the heterologous polynucleotides in the electrophoresis medium (e.g., further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

The term "purified" does not require absolute purity; rather, it is intended as a relative definition. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. As an example, purification from 0.1 % concentration to 10 % concentration is two orders of magnitude. To illustrate, individual cDNA clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. The sequences obtained from these clones could not be obtained directly either from the library or from total human DNA. The cDNA clones are not naturally occurring as such, but rather are obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The conversion of mRNA into a cDNA library involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection. Thus, creating a cDNA library from messenger RNA and subsequently isolating individual clones from that library results in an approximately 10^4-10^6 fold purification of the native message.

The term "purified" is further used herein to describe a polypeptide or polynucleotide of the invention which has been separated from other compounds including, but not limited to, polypeptides or polynucleotides, carbohydrates, lipids, etc. The term "purified" may be used to specify the separation of monomeric polypeptides of the invention from oligomeric forms such as homo- or hetero-dimers, trimers, etc. The term "purified" may also be used to specify the separation of covalently closed polynucleotides from linear polynucleotides. A polynucleotide is substantially pure when at least about 50%, preferably 60 to 75% of a sample exhibits a single polynucleotide sequence and conformation (linear versus covalently closed). A substantially pure polypeptide or polynucleotide typically comprises about 50%, preferably 60 to 90% weight/weight of a polypeptide or polynucleotide sample, respectively, more usually about 95%, and preferably is over about 99% pure. Polypeptide and polynucleotide purity, or homogeneity, is indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single band upon staining the gel. For certain purposes higher resolution can be provided by using HPLC or other means well known in the art. As an alternative embodiment, purification of the polypeptides and polynucleotides of the present invention may be expressed as "at least" a percent purity relative to heterologous polypeptides and polynucleotides (DNA, RNA or both). As a preferred embodiment, the polypeptides and polynucleotides of the present invention are at least; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 96%, 98%, 99%, or 100% pure relative to heterologous polypeptides and polynucleotides, respectively. As a further preferred embodiment, the polypeptides and polynucleotides have a purity ranging from any number, to the thousandth position, between 90% and 100% (e.g., a polypeptide or polynucleotide at least 99.995% pure) relative to either heterologous polypeptides or polynucleotides, respectively, or as a weight/weight ratio relative to all compounds and molecules other than those existing in the carrier. Each number representing a percent purity, to the thousandth position, may be claimed as individual species of purity.

As used interchangeably herein, the terms "nucleic acid molecule(s)", "oligonucleotide(s)", and "polynucleotide(s)" include RNA or DNA (either single or double stranded, coding, complementary or antisense), or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form (although each of the above species may be particularly specified). The term "nucleotide" is used herein as an adjective to describe molecules comprising RNA, DNA, or RNA/DNA hybrid sequences of any length in single-stranded or duplex form. More precisely, the expression "nucleotide sequence" encompasses the nucleic material itself and is thus not restricted to the sequence information (i.e. the succession of letters chosen among the four base letters) that biochemically characterizes a specific DNA or RNA molecule. The term "nucleotide" is also used herein as a noun to refer to individual nucleotides or varieties of nucleotides, meaning a molecule, or individual unit in a larger nucleic acid molecule, comprising a purine or pyrimidine, a ribose or deoxyribose sugar moiety, and a phosphate group, or phosphodiester linkage in the case of nucleotides within an oligonucleotide or polynucleotide. The term

"nucleotide" is also used herein to encompass "modified nucleotides" which comprise at least one modifications such as (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar. For examples of analogous linking groups, purine, pyrimidines, and sugars see for example PCT publication No. WO 95/04064, which disclosure is hereby incorporated by reference in its entirety. Preferred modifications of the present invention include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v) ybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art. Methylenemethylimino linked oligonucleosides as well as mixed backbone compounds having, may be prepared as described in U.S. Pat. Nos. 5,378,825; 5,386,023; 5,489,677; 5,602,240; and 5,610,289, which disclosures are hereby incorporated by reference in their entireties. Formacetal and thioformacetal linked oligonucleosides may be prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564, which disclosures are hereby incorporated by reference in their entireties. Ethylene oxide linked oligonucleosides may be prepared as described in U.S. Pat. No. 5,223,618, which disclosure is hereby incorporated by reference in its entirety. Phosphinate oligonucleotides may be prepared as described in U.S. Pat. No. 5,508,270, which disclosure is hereby incorporated by reference in its entirety. Alkyl phosphonate oligonucleotides may be prepared as described in U.S. Pat. No. 4,469,863, which disclosure is hereby incorporated by reference in its entirety. 3'-Deoxy-3'-methylene phosphonate oligonucleotides may be prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050 which disclosures are hereby incorporated by reference in their entireties. Phosphoramidite oligonucleotides may be prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878 which disclosures are hereby incorporated by reference in their entireties. Alkylphosphonothioate oligonucleotides may be prepared as described in published PCT applications WO 94/17093 and WO 94/02499 which disclosures are hereby incorporated by reference in their entireties. 3'-Deoxy-3'-amino phosphoramidate oligonucleotides may be prepared as described in U.S. Pat. No. 5,476,925, which disclosure is hereby incorporated by reference in its entirety. Phosphotriester oligonucleotides may be prepared as described in U.S. Pat. No. 5,023,243, which disclosure is hereby incorporated by reference in its entirety. Borano phosphate oligonucleotides

may be prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198 which disclosures are hereby incorporated by reference in their entireties.

The term "upstream" is used herein to refer to a location that is toward the 5' end of the polynucleotide from a specific reference point.

The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another by virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds (See Stryer, 1995, which disclosure is hereby incorporated by reference in its entirety).

The terms "complementary" or "complement thereof" are used herein to refer to the sequences of polynucleotides that are capable of forming Watson & Crick base pairing with another specified polynucleotide throughout the entirety of the complementary region. For the purpose of the present invention, a first polynucleotide is deemed to be complementary to a second polynucleotide when each base in the first polynucleotide is paired with its complementary base. Complementary bases are, generally, A and T (or A and U), or C and G. "Complement" is used herein as a synonym from "complementary polynucleotide", "complementary nucleic acid" and "complementary nucleotide sequence". These terms are applied to pairs of polynucleotides based solely upon their sequences and not any particular set of conditions under which the two polynucleotides would actually bind. Unless otherwise stated, all complementary polynucleotides are fully complementary on the whole length of the considered polynucleotide.

The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymer of amino acids without regard to the length of the polymer; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude chemical or post-expression modifications of the polypeptides of the invention, although chemical or post-expression modifications of these polypeptides may be included excluded as specific embodiments. Therefore, for example, modifications to polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term polypeptide. Further, polypeptides with these modifications may be specified as individual species to be included or excluded from the present invention. The natural or other chemical modifications, such as those listed in examples above can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment

of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. [See, for instance Creighton (1993); Seifter *et al.*, (1990); Rattan *et al.*, (1992)]. Also included within the definition are polypeptides which contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

As used herein, the terms "recombinant polynucleotide" and "polynucleotide construct" are used interchangeably to refer to linear or circular, purified or isolated polynucleotides that have been artificially designed and which comprise at least two nucleotide sequences that are not found as contiguous nucleotide sequences in their initial natural environment. In particular, these terms mean that the polynucleotide or cDNA is adjacent to "backbone" nucleic acid to which it is not adjacent in its natural environment. Additionally, to be "enriched" the cDNA will represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid backbone molecules. Backbone molecules according to the present invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. Preferably, the enriched cDNA represent 15% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. More preferably, the enriched cDNA represent 50% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. In a highly preferred embodiment, the enriched cDNA represent 90% or more (including any number between 90 and 100%, to the thousandth position, e.g., 99.5%) # of the number of nucleic acid inserts in the population of recombinant backbone molecules.

The term "recombinant polypeptide" is used herein to refer to polypeptides that have been artificially designed and which comprise at least two polypeptide sequences that are not found as contiguous polypeptide sequences in their initial natural environment, or to refer to polypeptides which have been expressed from a recombinant polynucleotide.

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A sequence which is "operably linked" to a regulatory sequence such as a promoter means that said regulatory element is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the nucleic acid of interest. For

instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence.

As used herein, the term "non-human animal" refers to any non-human animal, including insects, birds, rodents and more usually mammals. Preferred non-human animals include: primates; farm animals such as swine, goats, sheep, donkeys, cattle, horses, chickens, rabbits; and rodents, preferably rats or mice. As used herein, the term "animal" is used to refer to any species in the animal kingdom, preferably vertebrates, including birds and fish, and more preferable a mammal. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

The term "domain" refers to an amino acid fragment with specific biological properties. This term encompasses all known structural and linear biological motifs. Examples of such motifs include but are not limited to leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal peptides which direct the secretion of proteins, sites for post-translational modification, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

As used herein, the term "ligand" refers to a compound that binds to a receptor. Preferred "ligand" is a naturally occurring polypeptide or fragment thereof. Further preferred is said polypeptide, wherein binding of said polypeptide or fragment thereof to receptor elicits a biological response comprised of biological activities. Further preferred is said polypeptide, wherein said polypeptide is comprised C-terminally of C1q homology globular domain. Further preferred is said polypeptide, wherein said polypeptide is selected from the group of polypeptides comprising Table 2. Most preferably, said polypeptide is APM1.

As used herein, the terms "globular ACRP30", "gACRP30" or "gACRP30 polypeptide fragment" refer to a fragment of ACRP30 comprised of the C-terminal C1q homology globular domain of ACRP30. As used herein, the terms "globular APM1", "gAPM1" or "gAPM1 polypeptide fragment" refer to a fragment of APM1 comprised of the C-terminal C1q homology globular domain of APM1. As used herein, the terms "globular LIGAND", "gLIGAND" or "gLIGAND polypeptide fragment" refer to a fragment of LIGAND comprised of the C-terminal C1q homology globular domain of LIGAND.

As used herein, the term "receptor" refers to a polypeptide to which a "ligand" binds and through which said "ligand" elicits a biological response comprised of biological activities. Said receptor is preferably FRADJ and/or CRYPTIC of the present invention. By "receptor activation" is intended "ligand"-mediated alteration of said receptor polypeptide, wherein said alteration is selected from but not limited to the group consisting of receptor alterations associated with said biological response.

As used herein, the term "AGONIST" refers to naturally occurring and synthetic compounds capable of inducing, enhancing, or potentiating a biological response comprised of biological activities.

As used herein, the term "ANTAGONIST" refers to naturally occurring and synthetic compounds capable of inhibiting a biological response, inhibiting the induction of a biological response, or inhibiting the potentiation of a biological response, wherein said biological response is comprised of biological activities.

Without being limited by theory, the compounds/polypeptides of the invention are capable of modulating the partitioning of dietary lipids between the liver and peripheral tissues, and are thus believed to treat "diseases involving the partitioning of dietary lipids between the liver and peripheral tissues. " The term "peripheral tissues" is meant to include muscle and adipose tissue. In preferred embodiments, the compounds/polypeptides of the invention partition the dietary lipids toward or away from the muscle. In alternative preferred embodiments, the dietary lipids are partitioned toward or away from the adipose tissue. In other preferred embodiments, the dietary lipids are partitioned toward or away from the liver. In yet other preferred embodiments, the compounds/polypeptides of the invention increase or decrease the oxidation of dietary lipids, preferably free fatty acids (FFA) by the muscle. Dietary lipids include, but are not limited to triglycerides and free fatty acids.

Preferred diseases believed to involve the partitioning of dietary lipids include obesity-related diseases and disorders such as obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other disorders of the invention include disorders associated with excessive weight loss such as cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

The term "heterologous", when used herein, is intended to designate any polypeptide or polynucleotide other than a FRADJ and/or CRYPTIC polypeptide or a polynucleotide encoding a FRADJ and/or CRYPTIC polypeptide of the present invention.

The terms "comprising", "consisting of" and "consisting essentially of" may be interchanged for one another throughout the instant application, although each retains its normal definition. The term "having" has the same meaning as "comprising" and may be replaced with either the term "consisting of" or "consisting essentially of".

Unless otherwise specified in the application, nucleotides and amino acids of polynucleotides and polypeptides respectively of the present invention are contiguous and not interrupted by heterologous sequences.

Identity Between Nucleic Acids Or Polypeptides

The terms "percentage of sequence identity" and "percentage homology" are used interchangeably herein to refer to comparisons among polynucleotides and polypeptides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Homology is evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, CLUSTALW, FASTDB (Pearson and Lipman, 1988; Altschul *et al.*, 1990; Thompson *et al.*, 1994; Higgins *et al.*, 1996; Altschul *et al.*, 1990; Altschul *et al.*, 1993; Brutlag *et al*, 1990), the disclosures of which are incorporated by reference in their entireties.

In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") which is well known in the art (see, e.g., Karlin and Altschul, 1990; Altschul *et al.*, 1990, 1993, 1997), the disclosures of which are incorporated by reference in their entireties. In particular, five specific BLAST programs are used to perform the following task:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet *et al.*,

1992; Henikoff and Henikoff, 1993), the disclosures of which are incorporated by reference in their entireties. Less preferably, the PAM or PAM250 matrices may also be used (see, e.g., Schwartz and Dayhoff, eds., 1978), the disclosure of which is incorporated by reference in its entirety. The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (see, e.g., Karlin and Altschul, 1990), the disclosure of which is incorporated by reference in its entirety. The BLAST programs may be used with the default parameters or with modified parameters provided by the user.

Another preferred method for determining the best overall match between a query nucleotide sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.* (1990), the disclosure of which is incorporated by reference in its entirety. In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by first converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty= 1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score= 1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is 35 shorter. If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3'ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using 10, the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only nucleotides outside the 5' and 3' nucleotides of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 nucleotide subject sequence is aligned to a 100 nucleotide query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 nucleotides at 5' end. The 10 unpaired nucleotides represent 10% of the sequence (number of nucleotides at the 5' and 3' ends not matched/total number of nucleotides in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90

nucleotides were perfectly matched the final percent identity would be 90%. In another example, a 90 nucleotide subject sequence is compared with a 100 nucleotide query sequence. This time the deletions are internal deletions so that there are no nucleotides on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only nucleotides 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected. No other manual corrections are made for the purposes of the present invention.

Another preferred method for determining the best overall match between a query amino acid sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al*. (1990). In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty= 1, Joining Penalty=20, Randomization Group25Length=0, Cutoff Score= 1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. If the subject sequence is shorter than the query sequence due to N-or C-terminal deletions, not because of internal deletions, the results, in percent identity, must be manually corrected. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C- terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query amino acid residues outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100-residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not match/align with the first residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90-residue subject sequence is compared with a 100-residue query

sequence. This time the deletions are internal so there are no residues at the N- or C-termini of the subject sequence, which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected. No other manual corrections are made for the purposes of the present invention.

The term "percentage of sequence similarity" refers to comparisons between polypeptide sequences and is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which an identical or equivalent amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence similarity. Similarity is evaluated using any of the variety of sequence comparison algorithms and programs known in the art, including those described above in this section. Equivalent amino acid residues are defined herein in the "Mutated polypeptides" section.

Polynucleotides of the invention

The present invention provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) the full length sequence of SEQ ID NO:1; (b) a sequence of SEQ ID NO:1 encoding full length FRADJ of SEQ ID NO:2; (c) a sequence of SEQ ID NO:1 encoding full length FRADJ of SEQ ID NO:2 absent the N-terminal Met; (d) a sequence of SEQ ID NO:1 encoding mature FRADJ protein of SEQ ID NO:2 lacking signal peptide; (e) a sequence of SEQ ID NO:1 encoding the extracellular domain of FRADJ protein of SEQ ID NO:2; (f) a sequence of SEQ ID NO:1 encoding the transmembrane domain of FRADJ protein of SEQ ID NO:2; (g) a sequence of SEQ ID NO:1 encoding the intracellular domain of FRADJ protein of SEQ ID NO:2; (h) a sequence of SEQ ID NO:1 encoding the LIGAND binding domain of FRADJ protein of SEQ ID NO:2; (i) a sequence of SEQ ID NO:1 encoding FRADJ polypeptide of protein of SEQ ID NO:2 wherein said FRADJ polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (j) the variant polynucleotides of any of the polynucleotides of (a)-(i); (k) the polynucleotides comprising a nucleotide sequence of (a)-(j), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (l) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (k). The invention further provides for fragments of the nucleic acid molecules of (a)-(l) described above.

The present invention provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) the full length sequence of SEQ ID NO:3; (b) a sequence of SEQ ID NO:3 encoding full length CRYPTIC of SEQ ID NO:4; (c) a sequence of SEQ ID NO:3 encoding full length CRYPTIC of SEQ ID NO:4 absent the N-terminal Met; (d) a sequence of SEQ ID NO:3 encoding mature CRYPTIC protein of SEQ ID NO:4 lacking signal peptide; (e) a sequence of SEQ ID NO:3 encoding the extracellular domain of CRYPTIC protein of SEQ ID NO:4; (f) a sequence of SEQ ID NO:3 encoding the transmembrane domain of CRYPTIC protein of SEQ ID NO:4; (g) a sequence of SEQ ID NO:3 encoding the intracellular domain of CRYPTIC protein of SEQ ID NO:4; (h) a sequence of SEQ ID NO:3 encoding the LIGAND binding domain of CRYPTIC protein of SEQ ID NO:4; (i) a sequence of SEQ ID NO:3 encoding CRYPTIC polypeptide of protein of SEQ ID NO:4 wherein said CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (j) the variant polynucleotides of any of the polynucleotides of (a)-(i); (k) the polynucleotides comprising a nucleotide sequence of (a)-(j), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (l) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (k). The invention further provides for fragments of the nucleic acid molecules of (a)-(l) described above.

Further embodiments of the invention include a purified or isolated polynucleotide that comprise, consist of, or consist essentially of a nucleotide sequence at least 70% identical, more preferably at least 75%, and even more preferably at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical, to any of the nucleotide sequences in (a)-(l) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a)-(l) above.

The present invention additionally provides a purified or isolated polynucleotide comprising, consisting of, or consisting essentially of a nucleotide sequence selected from the group consisting of: (a) a polynucleotide encoding full length FRADJ or CRYPTIC of SEQ ID NO:2 or 4; (b) a polynucleotide encoding full length FRADJ or CRYPTIC of SEQ ID NO:2 or 4 absent the N-terminal Met; (c) a polynucleotide encoding mature FRADJ or CRYPTIC protein of SEQ ID NO:2 or 4 lacking signal peptide; (d) a polynucleotide encoding the extracellular domain of FRADJ or CRYPTIC protein of SEQ ID NO:2 or 4; (e) a polynucleotide encoding the transmembrane domain of FRADJ or CRYPTIC protein of SEQ ID NO:2 or 4; (f) a polynucleotide encoding the intracellular domain of FRADJ or CRYPTIC protein of SEQ ID NO:2 or 4; (g) a polynucleotide encoding the LIGAND binding domain of FRADJ or CRYPTIC protein of SEQ ID NO:2 or 4; (h) a polynucleotide encoding FRADJ or CRYPTIC polypeptide of protein of SEQ ID NO:2 or 4 wherein said FRADJ and/or CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the variant polynucleotides of any of the polynucleotides of (a)-(h); (j) the polynucleotides comprising a nucleotide

sequence of (a)-(i), wherein the polynucleotide is single stranded, double stranded, or a portion is single stranded and a portion is double stranded; (k) the polynucleotides comprising a nucleotide sequence complementary to any of the single stranded polynucleotides of (j). The invention further provides for fragments of the nucleic acid molecules of (a)-(k) described above.

Further embodiments of the invention include a purified or isolated polynucl eotide that comprise, consist of, or consist essentially of a nucleotide sequence at least 70% identical, more preferably at least 75%, and even more preferably at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical, to any of the nucleotide sequences in (a)-(k) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a)-(k) above.

The present invention concerns FRADJ and/or CRYPTIC genomic and cDNA sequences. The present invention encompasses FRADJ and/or CRYPTIC genes, polynucleotides comprising FRADJ and/or CRYPTIC genomic and cDNA sequences, as well as fragments and variants thereof. These polynucleotides may be purified, isolated, or recombinant.

Also encompassed by the present invention are allelic variants, orthologs, splice variants, and/or species homologues of the FRADJ and/or CRYPTIC genes. Procedures known in the art can be used to obtain full-length genes and cDNA, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologues of genes and cDNA corresponding to a nucleotide sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3, using information from the sequences disclosed herein. For example, allelic variants, orthologs and/or species homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue using any technique known to those skilled in the art including those described into the section entitled "To find similar sequences".

In a specific embodiment, the polynucleotides of the invention are at least 15, 30, 50, 100, 125, 500, or 1000 continuous nucleotides. In another embodiment, the polynucleotides are less than or equal to 300kb, 200kb, 100kb, 50kb, 10kb, 7.5kb, 5kb, 2.5kb, 2kb, 1.5kb, or 1kb in length. In a further embodiment, polynucleotides of the invention comprise a portion of th e coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 75, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 naturally occurring genomic flanking gene(s).

cDNA sequences of the invention

Another object of the invention is a purified, isolated, or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3,

complementary sequences thereto, and fragments thereof. Moreover, preferred polynucleotides of the invention include purified, isolated, or recombinant FRADJ and/or CRYPTIC cDNA consisting of, consisting essentially of, or comprising a sequence selected from the group consisting of SEQ ID NO:1or 3.

Accordingly, the coding sequence (CDS) or open reading frame (ORF) of each cDNA of the invention refers to the nucleotide sequence beginning with the first nucleotide of the start codon and ending with the last nucleotide of the stop codon. Similarly, the 5'untranslated region (or 5'UTR) of each cDNA of the invention refers to the nucleotide sequence starting at nucleotide 1 and ending at the nucleotide immediately 5' to the first nucleotide of the start codon. The 3'untranslated region (or 3'UTR) of each cDNA of the invention refers to the nucleotide sequence starting at the nucleotide immediately 3' to the last nucleotide of the stop codon and ending at the last nucleotide of the cDNA.

Untranslated Regions

In addition, the invention concerns a purified, isolated, and recombinant nucleic acid comprising a nucleotide sequence selected from the group consisting of the 5'UTRs of sequences of SEQ ID NO:1 or 3, sequences complementary thereto, and allelic variants thereof. The invention also concerns a purified, isolated, and recombinant nucleic acid comprising a nucleotide sequence selected from the group consisting of the 3'UTRs of sequences of SEQ ID NO:1 or 3, sequences complementary thereto, and allelic variants thereof.

These polynucleotides may be used to detect the presence of FRADJ and/or CRYPTIC mRNA species in a biological sample using either hybridization or RT-PCR techniques well known to those skilled in the art.

In addition, these polynucleotides may be used as regulatory molecules able to affect the processing and maturation of the polynucleotide including them (either a FRADJ and/or CRYPTIC polynucleotide or an heterologous polynucleotide), preferably the localization, stability and/or translation of said polynucleotide including them (for a review on UTRs see Decker and Parker, 1995, Derrigo *et al.*, 2000). In particular, 3'UTRs may be used in order to control the stability of heterologous mRNAs in recombinant vectors using any methods known to those skilled in the art including Makrides (1999), US Patents 5,925,56; 5,807,7 and 5,756,264, which disclosures are hereby incorporated by reference in their entireties.

Coding Sequences

Another object of the invention is an isolated, purified or recombinant polynucleotide comprising the coding sequence of a sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3 and variants thereof.

A further object of the invention is an isolated, purified or recombinant polynucleotide encoding a polypeptide comprising a sequence selected from the group consisting of sequences of SEQ ID NO:2 or 4 and allelic variants thereof.

It will be appreciated that should the extent of the coding sequence differ from that indicated in the appended sequence listing as a result of a sequencing error, reverse transcription or amplification error, mRNA splicing, post-translational modification of the encoded protein, enzymatic cleavage of the encoded protein, or other biological factors, one skilled in the art would be readily able to identify the extent of the coding sequences in the sequences of SEQ ID NO:1 or 3. Accordingly, the scope of any claims herein relating to nucleic acids containing the coding sequence of one of SEQ ID NO:1 or 3 is not to be construed as excluding any readily identifiable variations from or equivalents to the coding sequences described in the appended sequence listing. Similarly, should the extent of the polypeptides differ from those indicated in the appended sequence listing as a result of any of the preceding factors, the scope of claims relating to polypeptides comprising the amino acid sequence of the polypeptides of SEQ ID NO:2 or 4 is not to be construed as excluding any readily identifiable variations from or equivalents to the sequences described in the appended sequence listing.

The above disclosed polynucleotides that contain the coding sequence of the FRADJ and/or CRYPTIC genes may be expressed in a desired host cell or a desired host organism, when this polynucleotide is placed under the control of suitable expression signals. The expression signals may be either the expression signals contained in the regulatory regions in the FRADJ and/or CRYPTIC genes of the invention or in contrast the signals may be exogenous regulatory nucleic sequences. Such a polynucleotide, when placed under the suitable expression signals, may also be inserted in a vector for its expression and/or amplification.

Further included in the present invention are polynucleotides encoding the polypeptides of the present invention that are fused in frame to the coding sequences for additional heterologous amino acid sequences. Also included in the present invention are nucleic acids encoding polypeptides of the present invention together with additional, non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, vector sequence, sequences used for purification, probing, or priming. For example, heterologous sequences include transcribed, untranslated sequences that may play a role in transcription, and mRNA processing, for example, ribosome binding and stability of mRNA. The heterologous sequences may alternatively comprise additional coding sequences that provide additional functionalities. Thus, a nucleotide sequence encoding a polypeptide may be fused to a tag sequence, such as a sequence encoding a peptide that facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN), among others, many of which are commercially available. For instance, hexa-histidine provides for convenient purification of the fusion protein (See Gentz *et al.*, 1989), the disclosure of which is incorporated by reference in its entirety. The

"HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein (See Wilson *et al.*, 1984), the disclosure of which is incorporated by reference in its entirety. As discussed below other such fusion proteins include the FRADJ and/or CRYPTIC protein fused to Fc at the N- or C-terminus.

Suitable recombinant vectors that contain a polynucleotide such as described herein are disclosed elsewhere in the specification. Expression vectors encoding FRADJ and/or CRYPTIC polypeptides or fragments thereof are described in the section entitled "Preparation of the Polypeptides".

Regulatory Sequences of the Invention

As mentioned, the genomic sequence of FRADJ and/or CRYPTIC genes contains regulatory sequences in the non-coding 5'-flanking region and possibly in the non-coding 3'-flanking region that border the FRADJ and/or CRYPTIC coding regions containing the exons of these genes.

Polynucleotides derived from FRADJ and/or CRYPTIC 5' and 3' regulatory regions are useful in order to detect the presence of at least a copy of a genomic nucleotide sequence of the FRADJ and/or CRYPTIC gene or a fragment thereof in a test sample.

Preferred regulatory sequences

Polynucleotides carrying the regulatory elements located at the 5' end and at the 3' end of FRADJ and/or CRYPTIC coding regions may be advantageously used to control the transcriptional and translational activity of a heterologous polynucleotide of interest.

Thus, the present invention also concerns a purified or isolated nucleic acid comprising a polynucleotide which is selected from the group consisting of the 5' and 3' FRADJ and/or CRYPTIC regulatory regions, sequences complementary thereto, regulatory active fragments and variants thereof. The invention also pertains to a purified or isolated nucleic acid comprising a polynucleotide having at least 95% nucleotide identity with a polynucleotide selected from the group consisting of FRADJ and/or CRYPTIC 5' and 3' regulatory regions, advantageously 99% nucleotide identity, preferably 99.5% nucleotide identity and most preferably 99.8% nucleotide identity with a polynucleotide selected from the group consisting of FRADJ and/or CRYPTIC 5' and 3' regulatory regions, sequences complementary thereto, variants and regulatory active fragments thereof.

Another object of the invention consists of purified, isolated or recombinant nucleic acids comprising a polynucleotide that hybridizes, under the stringent hybridization conditions defined herein, with a polynucleotide selected from the group consisting of the nucleotide sequences of FRADJ and/or CRYPTIC 5'- and 3' regulatory regions, sequences complementary thereto, variants and regulatory active fragments thereof.

Preferred fragments of 5' regulatory regions have a length of about 1500 or 1000 nucleotides, preferably of about 500 nucleotides, more preferably about 400 nucleotides, even more preferably 300 nucleotides and most preferably about 200 nucleotides.

Preferred fragments of 3' regulatory regions are at least 20, 50, 100, 150, 200, 300 or 400 bases in length.

"Regulatory active" polynucleotide derivatives of the 5' regulatory region are polynucleotides comprising or alternatively consisting of a fragment of said polynucleotide which is functional as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide in a recombinant cell host. It could act either as an enhancer or as a repressor. For the purpose of the invention, a nucleic acid or polynucleotide is "functional" as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide if said regulatory polynucleotide contains nucleotide sequences that contain transcriptional and translational regulatory information, and such sequences are "operably linked" to nucleotide sequences that encode the desired polypeptide or the desired polynucleotide.

The regulatory polynucleotides of the invention may be prepared from the nucleotide sequence of FRADJ and/or CRYPTIC genomic or cDNA sequence, for example, by cleavage using suitable restriction enzymes, or by PCR. The regulatory polynucleotides may also be prepared by digestion of a FRADJ and/or CRYPTIC gene containing genomic clone by an exonuclease enzyme, such as Bal31 (Wabiko *et al.*, 1986), the disclosure of which is incorporated by reference in its entirety. These regulatory polynucleotides can also be prepared by nucleic acid chemical synthesis, as described elsewhere in the specification.

The regulatory polynucleotides according to the invention may be part of a recombinant expression vector that may be used to express a coding sequence in a desired host cell or host organism. The recombinant expression vectors according to the invention are described elsewhere in the specification.

Preferred 5'-regulatory polynucleotide of the invention include 5'-UTRs of FRADJ and/or CRYPTIC cDNA, or regulatory active fragments or variants thereof. More preferred 5'-regulatory polynucleotides of the invention include sequences selected from the group consisting of 5'-UTRs of sequences of SEQ ID NO:1 or 3, 5'-UTRs of clones inserts of the deposited clone pool, regulatory active fragments and variants thereof.

Preferred 3'-regulatory polynucleotide of the invention include 3'-UTRs of FRADJ and/or CRYPTIC cDNA, or regulatory active fragments or variants thereof. More preferred 3'-regulatory polynucleotides of the invention include sequences selected from the group consisting of 3'-UTRs of sequences of SEQ ID NO:1 or 3, 3'-UTRs of clones inserts of the deposited clone pool, regulatory active fragments and variants thereof.

A further object of the invention consists of a purified or isolated nucleic acid comprising:

a) a polynucleotide comprising a 5' regulatory nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence comprising a polynucleotide of a FRADJ and/or CRYPTIC 5' regulatory region or a complementary sequence thereto;

(ii) a nucleotide sequence comprising a polynucleotide having at least 95% of nucleotide identity with the nucleotide sequence of a FRADJ and/or CRYPTIC 5' regulatory region or a complementary sequence thereto;

(iii) a nucleotide sequence comprising a polynucleotide that hybridizes under stringent hybridization conditions with the nucleotide sequence of a FRADJ and/or CRYPTIC 5' regulatory region or a complementary sequence thereto; and

(iv) a regulatory active fragment or variant of the polynucleotides in (i), (ii) and (iii);

b) a nucleic acid molecule encoding a desired polypeptide or a nucleic acid molecule of interest, said nucleic acid molecule is operably linked to the polynucleotide defined in (a); and

c) optionally, a polynucleotide comprising a 3'- regulatory polynucleotide, preferably a 3'-regulatory polynucleotide of a FRADJ and/or CRYPTIC gene.

In a specific embodiment, the nucleic acid defined above includes the 5'-UTR of a FRADJ and/or CRYPTIC cDNA, or a regulatory active fragment or variant thereof.

In a second specific embodiment, the nucleic acid defined above includes the 3'-UTR of a FRADJ and/or CRYPTIC cDNA, or a regulatory active fragment or variant thereof.

The regulatory polynucleotide of the 5' regulatory region, or its regulatory active fragments or variants, is operably linked at the 5'-end of the nucleic acid molecule encoding the desired polypeptide or nucleic acid molecule of interest.

The regulatory polynucleotide of the 3' regulatory region, or its regulatory active fragments or variants, is advantageously operably linked at the 3'-end of the nucleic acid molecule encoding the desired polypeptide or nucleic acid molecule of interest.

The desired polypeptide encoded by the above-described nucleic acid may be of various nature or origin, encompassing proteins of prokaryotic viral or eukaryotic origin. Among the polypeptides expressed under the control of a FRADJ and/or CRYPTIC regulatory region include bacterial, fungal or viral antigens. Also encompassed are eukaryotic proteins such as intracellular proteins, such as "house

keeping" proteins, membrane-bound proteins, such as mitochondrial membrane-bound proteins and cell surface receptors, and secreted proteins such as endogenous mediators such as cytokines. The desired polypeptide may be an heterologous polypeptide or a FRADJ and/or CRYPTIC protein, especially a protein with an amino acid sequence selected from the group consisting of sequences of SEQ ID NO:2 or 4, fragments and variants thereof.

The desired nucleic acids encoded by the above-described polynucleotide, usually an RNA molecule, may be complementary to a desired coding polynucleotide, for example to a FRADJ and/or CRYPTIC coding sequence, and thus useful as an antisense polynucleotide. Such a polynucleotide may be included in a recombinant expression vector in order to express the desired polypeptide or the desired nucleic acid in host cell or in a host organism. Suitable recombinant vectors that contain a polynucleotide such as described herein are disclosed elsewhere in the specification.

Polynucleotide Variants

The invention also relates to variants of the polynucleotides described herein and fragments thereof. "Variants" of polynucleotides, as the term is used herein, are polynucleotides that differ from a reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical. The present invention encompasses both allelic variants and degenerate variants.

Allelic Variant

A variant of a polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (see Lewin, 1990), the disclosure of which is incorporated by reference in its entirety. Diploid organisms may be homozygous or heterozygous for an allelic form. Non-naturally occurring variants of the polynucleotide may be made by art-known mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Degenerate Variant

In addition to the isolated polynucleotides of the present invention, and fragments thereof, the invention further includes polynucleotides that comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode a FRADJ and/or CRYPTIC polypeptide of the present invention. These polynucleotide variants are referred to as "degenerate variants" throughout the instant application. That is, all possible polynucleotide sequences that encode the FRADJ and/or CRYPTIC polypeptides of the present invention are completed. This includes the genetic code and species-specific codon preferences known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above, for instance, to

optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by other mammalian or bacterial host cells).

Nucleotide changes present in a variant polynucleotide may be silent, which means that they do not alter the amino acids encoded by the polynucleotide. However, nucleotide changes may al so result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. In the context of the present invention, preferred embodiments are those in which the polynu cleotide variants encode polypeptides that retain substantially the same biological properties or activities as the FRADJ and/or CRYPTIC protein. More preferred polynucleotide variants are those containing conservative substitutions.

Similar Polynucleotides

Other embodiments of the present invention is a purified, isolated or recombinant polynucleotide which is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of sequences of SEQ ID NO:1 or 3 and clone inserts of the deposited clone pool. The above polynucleotides are included regardless of whether they encode a polypeptide having a FRADJ and/or CRYPTIC biological activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having FRADJ and/or CRYPTIC activity include, inter alia, isolating a FRADJ and/or CRYPTIC gene or allelic variants thereof from a DNA library, and detecting FRADJ and/or CRYPTIC mRNA expression in biological samples, suspected of containing FRADJ and/or CRYPTIC mRNA or DNA by Northern Blot or PCR analysis.

The present invention is further directed to polynucleotides having sequences at least 50%. 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identity to a polynucleotide selected from the group consisting of sequences of SEQ ID NO:1 or 3 and clone inserts of the deposited clone pool, where said polynucleotide do, in fact, encode a polypeptide having a FRADJ and/or CRYPTIC biological activity. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the polynucleotides at least 50%. 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a polynucleotide selected from the group consisting of sequences of SEQ ID NO:1 or 3 and clone inserts of the deposited clone pool will encode a polypeptide having biological activity. In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performin g the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants,

a reasonable number will also encode a polypeptide having biological activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below. By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the FRADJ and/or CRYPTIC polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted, inserted, or substituted with another nucleotide. The query sequence may be an entire sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool, or the ORF (open reading frame) of a polynucleotide sequence selected from said group, or any fragment specified as described herein.

Hybridizing Polynucleotides

In another aspect, the invention provides an isolated or purified nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to any polynucleotide of the present invention using any methods known to those skilled in the art including those disclosed herein and in particular in the "To find similar sequences" section. Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions, preferably at moderate or low stringency conditions as defined herein. Such hybridizing polynucleotides may be of at least 15,18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 200, 300, 500 or 1000 nucleotides in length.

A probe comprising at least 10 consecutive nucleotides from a FRADJ and/or CRYPTIC cDNA or fragment thereof is labeled with a detectable label such as a radioisotope or a fluorescent molecule. Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the cDNA or fragment thereof. More preferably, the probe comprises 20 to 30 consecutive nucleotides from the cDNA or fragment thereof. In some embodiments, the probe comprises more than 30 nucleotides from the cDNA or fragment thereof.

Techniques for labeling the probe are well known and include phosphorylation with polynucleotide kinase, nick translation, *in vitro* transcription, and non-radioactive techniques. The cDNA or genomic DNAs in the library are transferred to a nitrocellulose or nylon filter and denatured. After blocking of non-specific sites, the filter is incubated with the labeled probe for an amount of time sufficient to allow binding of the probe to cDNA or genomic DNAs containing a sequence capable of hybridizing thereto.

Of particular interest, are the polynucleotides hybridizing to any polynucleotide of the invention and encoding FRADJ and/or CRYPTIC polypeptides, particularly FRADJ and/or CRYPTIC polypeptides exhibiting a FRADJ and/or CRYPTIC biological activity.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a 5' complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

By varying the stringency of the hybridization conditions used to identify cDNA or genomic DNAs that hybridize to the detectable probe, cDNA or genomic DNAs having different levels of identity to the probe can be identified and isolated as described below.

Stringent conditions

"Stringent hybridization conditions" are defined as conditions in which only nucleic acids having a high level of identity to the probe are able to hybridize to said probe. These conditions may be calculated as follows:

For probes between 14 and 70 nucleotides in length the melting temperature (Tm) is calculated using the formula: Tm=81.5+16.6(log (Na+))+0.41(fraction G+C)-(600/N) where N is the length of the probe.

If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation: Tm=81.5+16.6(log (Na+))+0.41(fraction G+C)-(0.63% formamide)-(600/N) where N is the length of the probe.

Prehybridization may be carried out in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100 µg denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100 µg denatured fragmented salmon sperm DNA, 50% formamide. The formulas for SSC and Denhardt's solutions are listed in Sambrook *et al.*, 1986.

Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to nucleic acids containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the hybridization may be carried out at 15-25°C below the Tm. For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 15-25°C below the Tm. Preferably, for hybridizations in 6X SSC, the hybridization is conducted at approximately 68°C. Preferably, for hybridizations in 50% formamide containing solutions, the hybridization is conducted at approximately 42°C.

Following hybridization, the filter is washed in 2X SSC, 0.1% SDS at room temperature for 15 minutes. The filter is then washed with 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour. Thereafter, the solution is washed at the hybridization temperature in 0.1X SSC, 0.5% SDS. A final wash is conducted in 0.1X SSC at room temperature.

Nucleic acids that have hybridized to the probe are identified by autoradiography or other conventional techniques.

Low and moderate conditions

Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. The above procedure may thus be modified to identify nucleic acids having decreasing levels of identity to the probe sequence. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a sodium concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of identity to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. cDNA or genomic DNAs which have hybridized to the probe are identified by autoradiography or other conventional techniques.

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Consequently, the present invention encompasses methods of isolating nucleic acids similar to the polynucleotides of the invention, comprising the steps of:

a) contacting a collection of cDNA or genomic DNA molecules with a detectable probe comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40 or 50 consecutive nucleotides of a sequence selected from the group consisting of the sequences of SEQ ID NO:1 or 3, the sequences of clones inserts of the deposited clone pool and sequences complementary thereto under stringent, moderate or low conditions which permit said probe to hybridize to at least a cDNA or genomic DNA molecule in said collection;

b) identifying said cDNA or genomic DNA molecule which hybridizes to said detectable probe; and

c) isolating said cDNA or genomic DNA molecule which hybridized to said probe.

PCR-based methods

In addition to the above described methods, other protocols are available to obtain homologous cDNA using FRADJ and/or CRYPTIC cDNA of the present invention or fragment thereof as outlined in the following paragraphs.

cDNA may be prepared by obtaining mRNA from the tissue, cell, or organism of interest using mRNA preparation procedures utilizing polyA selection procedures or other techniques known to those skilled in the art. A first primer capable of hybridizing to the polyA tail of the mRNA is hybridized to the mRNA and a reverse transcription reaction is performed to generate a first cDNA strand.

The term "capable of hybridizing to the polyA tail of said mRNA" refers to and embraces all primers containing stretches of thymidine residues, so-called oligo (dT) primers, that hybridize to the 3' end of eukaryotic poly(A)+ mRNAs to prime the synthesis of a first cDNA strand. Techniques for generating said oligo (dT) primers and hybridizing them to mRNA to subsequently prime the reverse transcription of said hybridized mRNA to Generate a first cDNA strand are well known to those skilled in the art and are described in Current Protocols in Molecular Biology, John Wiley and Sons, Inc. 1997 and Sambrook *et al.*, 1989. Preferably, said oligo (dT) primers are present in a large excess in order to allow the hybridization of all mRNA 3'ends to at least one oligo (dT) molecule. The priming and reverse transcription steps are preferably performed between 37°C and 55°C depending on the type of reverse transcriptase used. Preferred oligo(dT) primers for priming reverse transcription of mRNAs are oligonucleotides containing a stretch of thymidine residues of sufficient length to hybridize specifically to the polyA tail of mRNAs, preferably of 12 to 18 thymidine residues in length. More preferably, such oligo(T) primers comprise an additional sequence upstream of the poly(dT) stretch in order to allow the addition of a given sequence to the 5'end of all first cDNA strands which may then be used to facilitate subsequent manipulation of the cDNA. Preferably, this added sequence is 8 to 60 residues in length. For instance, the addition of a restriction site in 5' of cDNA facilitates subcloning of the obtained cDNA. Alternatively, such an added 5'end may also be used to design primers of PCR to specifically amplify cDNA clones of interest.

The first cDNA strand is then hybridized to a second primer. This second primer contains at least 10 consecutive nucleotides of a polynucleotide of the invention. Preferably, the primer comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides of a polynucleotide of the invention. In some embodiments, the primer comprises more than 30 nucleotides of a polynucleotide of the invention. If it is desired to obtain cDNA containing the full protein coding sequence, including the authentic translation initiation site, the second primer used contains sequences located upstream of the translation

initiation site. The second primer is extended to generate a second cDNA strand compl ementary to the first cDNA strand. Alternatively, RT-PCR may be performed as described above using primers from both ends of the cDNA to be obtained.

The double stranded cDNA made using the methods described above are isolated and cloned. The cDNA may be cloned into vectors such as plasmids or viral vectors capable of replicating in an appropriate host cell. For example, the host cell may be a bacterial, mammalian, avian, or insect cell.

Techniques for isolating mRNA, reverse transcribing a primer hybridi zed to mRNA to generate a first cDNA strand, extending a primer to make a second cDNA strand complementary to the first cDNA strand, isolating the double stranded cDNA and cloning the double stranded cDNA are well known to those skilled in the art and are described in *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. 1997 and Sambrook *et al.*, 1989.

Consequently, the present invention encompasses methods of making cDNA. In a first embodiment, the method of making a cDNA comprises the steps of

a) contacting a collection of mRNA molecules from human cells with a primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of the sequences complementary to SEQ ID NO:1 or 3 and sequences complementary to a clone insert of the deposited clone pool;

b) hybridizing said primer to an mRNA in said collection;

c) reverse transcribing said hybridized primer to make a first cDNA strand from said mRNA;

d) making a second cDNA strand complementary to said first cDNA strand; and

e) isolating the resulting cDNA comprising said first cDNA strand and said second cDNA strand.

Another embodiment of the present invention is a purified cDNA obtainable by the method of the preceding paragraph. In one aspect of this embodiment, the cDNA encodes at least a portion of a human polypeptide.

In a second embodiment, the method of making a cDNA comprises the steps of

a) contacting a collection of mRNA molecules from human cells with a first primer capable of hybridizing to the polyA tail of said mRNA;

b) hybridizing said first primer to said polyA tail;

c) reverse transcribing said mRNA to make a first cDNA strand;

d) making a second cDNA strand complementary to said first cDNA strand using at least one primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool; and

e) isolating the resulting cDNA comprising said first cDNA strand and said second cDNA strand.

In another aspect of this method the second cDNA strand is made by

a) contacting said first cDNA strand with a second primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool, and a third primer which sequence is fully included within the sequence of said first primer;

b) performing a first polymerase chain reaction with said second and third primers to generate a first PCR product;

c) contacting said first PCR product with a fourth primer, comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of said sequence selected from the group consisting of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool, and a fifth primer, which sequence is fully included within the sequence of said third primer, wherein said fourth and fifth hybridize to sequences within said first PCR product; and

d) performing a second polymerase chain reaction, thereby generating a second PCR product.

Alternatively, the second cDNA strand may be made by contacting said first cDNA strand with a second primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool, and a third primer which sequence is fully included within the sequence of said first primer and performing a polymerase chain reaction with said second and third primers to generate said second cDNA strand.

Alternatively, the second cDNA strand may be made by

a) contacting said first cDNA strand with a second primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool;

b) hybridizing said second primer to said first strand cDNA; and

c) extending said hybridized second primer to generate said second cDNA strand.

Another embodiment of the present invention is a purified cDNA obtainable by a method of making a cDNA of the invention. In one aspect of this embodiment, said cDNA encodes at least a portion of a human polypeptide.

Other protocols

Alternatively, other procedures may be used for obtaining homologous cDNA. In one approach, cDNA are prepared from mRNA and cloned into double stranded phagemids as follows. The cDNA library in the double stranded phagemids is then rendered single stranded by treatment with an endonuclease, such as the gene II product of the phage F1 and an exonuclease (Chang *et al.*, 1993, which disclosure is hereby incorporated by reference in its entirety). A biotinylated oligonucleotide comprising the sequence of a fragment of a known FRADJ and/or CRYPTIC cDNA, genomic DNA or fragment thereof is hybridized to the single stranded phagemids. Preferably, the fragment comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides of a sequence selected from the group consisting of the sequences of SEQ ID NO:1 or 3 and sequences of clone inserts of the deposited clone pool.

Hybrids between the biotinylated oligonucleotide and phagemids are isolated by incubating the hybrids with streptavidin coated paramagnetic beads and retrieving the beads with a magnet (Fry *et al,* 1992, which disclosure is hereby incorporated by reference in its entirety). Thereafter, the resulting phagemids are released from the beads and converted into double stranded DNA using a primer specific for the FRADJ and/or CRYPTIC cDNA or fragment used to design the biotinylated oligonucleotide. Alternatively, protocols such as the Gene Trapper kit (Gibco BRL), which disclosure is which disclosure is hereby incorporated by reference in its entirety, may be used. The resulting double stranded DNA is transformed into bacteria. Homologous cDNA to the FRADJ and/or CRYPTIC cDNA or fragment thereof sequence are identified by colony PCR or colony hybridization.

Any polynucleotide fragment of the invention may be used, and in particular those described in "Polynucleotide Fragments" can be used in a variety of ways, including, but not limited to, expressing the polypeptide in recombinant cells for use in screening assays for ANTAGONISTS and AGONISTS of its activity as well as to facilitate its purification for use in a variety of ways including, but not limited to screening assays for AGONISTS and ANTAGONISTS of its activity, diagnostic screens, and raising antibodies, as well as treatment and/or prevention of diseases and disorders disclosed herein.

The invention relates to the polynucleotides encoding gene polypeptides and variant polypeptide fragments thereof as described herein. These polynucleotides may be purified, isolated, and/or recombinant. Preferred gene polynucleotides of the invention are those that encode gene polypeptides of the invention have a biological activity as described and discussed herein.

Complementary Polynucleotides

The invention further provides isolated nucleic acid molecules having a nucleotide sequence fully complementary to any polynucleotide of the invention. The present invention encompasses a purified, isolated or recombinant polynucleotide having a nucleotide sequence complementary to a sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3, sequences of clone inserts of the deposited clone pool and fragments thereof. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping and for identifying FRADJ and/or CRYPTIC mRNA in a biological sample, for instance, by PCR or Northern blot analysis.

Polynucleotide Fragments

The recombinant polynucleotides encoding gene polypeptides can be used in a variety of ways, including, but not limited to, expressing the polypeptide in recombinant cells for use in screening assays for ANTAGONISTS and AGONISTS of its activity as well as to facilitate its purification for use in a variety of ways including, but not limited to screening assays for AGONISTS and ANTAGONISTS of its activity, diagnostic screens, and raising antibodies, as well as treatment and/or prevention of diseases and disorders disclosed herein.

The invention relates to the polynucleotides encoding gene polypeptides and variant polypeptide fragments thereof as described herein. These polynucleotides may be purified, isolated, and/or recombinant. Preferred gene polynucleotides of the invention are those that encode gene polypeptides of the invention have a biological activity as described and discussed herein.

Fragments

A polynucleotide fragment is a portion of a polynucleotide of the sequence listing or a sequence that encodes a specified polypeptide of the invention. Such fragments may be "free-standing", *i.e.* not part of or fused to other polynucleotides, or they may be comprised within another non-gene (heterologous) polynucleotide of which they form a part or region. However, several gene polynucleotide fragments may be comprised within a single polynucleotide.

In one aspect of this embodiment, the polynucleotide comprises at least 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895,

900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, 1000, 1005, 1010, 1015, 1020 consecutive nucleotides of a polynucleotide of the present invention.

In addition to the above preferred nucleic acid sizes, further preferred nucleic acids comprise at least 18 nucleotides, wherein "at least 18" is defined as any integer between 18 and an integer representing the 3' most nucleotide position of a polynucleotide as set forth in the sequence listing (SEQ ID NO:1 or 3) or elsewhere herein.

Further included as preferred polynucleotides of the present invention are nucleic acid fragments at least 18 nucleotides in length, as described above, that are further specified in terms of their 5' and 3' position. The 5' and 3' positions are represented by the position numbers set forth in the sequence listing below. For allelic and degenerate and other variants, position 1 is defined as the 5' most nucleotide of the ORF, i.e., the nucleotide "A" of the start codon (ATG) with the remaining nucleotides numbered consecutively. Therefore, every combination of a 5' and 3' nucleotide position that a polynucleotide of the present invention, at least 18 contiguous nucleotides in length, could occupy on an intact gene polynucleotide sequence of the present invention is included in the invention as an individual species. The polynucleotide fragments specified by 5' and 3' positions can be immediately envisaged and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specification.

It is noted that the above species of polynucleotide fragments of the present invention may alternatively be described by the formula "x to y"; where "x" equals the 5' most nucleotide position and "y" equals the 3' most nucleotide position of the polynucleotide; and further where "x" equals an integer between 1 and the number of nucleotides of the polynucleotide sequence of the present invention minus 18, and where "y" equals an integer between 19 and the number of nucleotides of the polynucleotide sequence of the present invention minus 18 nucleotides; and where "x" is an integer smaller then "y" by at least 18.

The present invention also provides for the exclusion of any species of polynucleotide fragments of the present invention specified by 5' and 3' positions or polynucleotides specified by size in nucleotides as described above. Any number of fragments specified by 5' and 3' positions or by size in nucleotides, as described above, may be excluded.

Each possible combination of "x" and "y" positions are included as a separate polynucleotide species of the invention. Moreover, the formula "x" to "y" may be modified as "'x1 – x2" to "y1 – y2"', wherein "x1 – x2" and "y1 – y2" represent positional ranges selected from any two nucleotide positions that "x" or "y" may occupy of the sequence listing. Alternative formulas include "'x1 – x2" to "y"' and "'x" to "y1 – y2"'.

The present invention also provides for the exclusion of any species of polynucleotide fragments of the present invention specified by 5' and 3' positions or polynucleotides specified by size in

nucleotides as described above. Any number of fragments specified by 5' and 3' positions or by size in nucleotides, as described above, may be excluded.

Methods of Making the Polynucleotides of the Invention

The present invention also comprises methods of making the polynucleotides of the invention, including the polynucleotides of SEQ ID NO:1 and/or 3, genomic DNA obtainable therefrom, or fragment thereof. These methods comprise sequentially linking together nucleotides to produce the nucleic acids having the preceding sequences. Polynucleotides of the invention may be synthesized either enzymatically using techniques well known to those skilled in the art including amplification or hybridization-based methods as described herein, or chemically.

A variety of chemical methods of synthesizing nucleic acids are known to those skilled in the art. In many of these methods, synthesis is conducted on a solid support. These included the 3' phosphoramidite methods in which the 3' terminal base of the desired oligonucleotide is immobilized on an insoluble carrier. The nucleotide base to be added is blocked at the 5' hydroxyl and activated at the 3' hydroxyl so as to cause coupling with the immobilized nucleotide base. Deblocking of the new immobilized nucleotide compound and repetition of the cycle will produce the desired polynucleotide. Alternatively, polynucleotides may be prepared as described in U.S. Patent No. 5,049,656, which disclosure is hereby incorporated by reference in its entirety. In some embodiments, several polynucleotides prepared as described above are ligated together to generate longer polynucleotides having a desired sequence.

Polypeptides of the Invention

The invention further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) the full length FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively; (b) the full length FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, absent the N-terminal Met; (c) mature FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, lacking signal peptide; (d) the extracellular domain of FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, (e) the transmembrane domain of FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, (f) the intracellular domain of FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, (g) a LIGAND binding fragment of FRADJ and CRYPTIC polypeptide of protein SEQ ID NO:2 and 4, respectively, (h) FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively, wherein said FRADJ and/or CRYPTIC polypeptide is of any one integer in length between 6 amino acids and 129 amino acids (full length) inclusive; (i) the epitope-bearing fragments of FRADJ and CRYPTIC polypeptide of protein of SEQ ID NO:2 and 4, respectively,; (j) the allelic variant polypeptides of any of the polypeptides of (a)-(i). The

invention further provides for fragments of the polypeptides of (a)-(i) above, such as those having biological activity or comprising biologically functional domain(s).

The present invention further includes polypeptides with an amino acid sequence with at least 70% similarity, and more preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to those polypeptides described in (a)-(i), as well as polypeptides having an amino acid sequence at least 70% identical, more preferably at least 75% identical, and still more preferably 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to those polypeptides described in (a)-(i). The invention further relates to methods of making the polypeptides of the present invention.

The term "FRADJ and/or CRYPTIC polypeptides" is used herein to embrace all of the proteins and polypeptides of the present invention. The present invention encompasses FRADJ and/or CRYPTIC polypeptides, including recombinant, isolated or purified FRADJ and/or CRYPTIC polypeptides consisting of, consisting essentially of, or comprising a sequence selected from the group consisting of SEQ ID NO:2 and/or 4, and the polypeptides encoded by human cDNA contained in the deposited clones. Other objects of the invention are polypeptides encoded by the polynucleotides of the invention as well as fusion polypeptides comprising such polypeptides.

Polypeptide Variants

The present invention further provides for FRADJ and/or CRYPTIC polypeptides encoded by allelic and splice variants, orthologs, and/or species homologues. Procedures known in the art can be used to obtain, allelic variants, splice variants, orthologs, and/or species homologues of polynucleotides encoding by polypeptides of the group consisting of SEQ ID NO:2 and 4, respectively, using information from the sequences disclosed herein.

The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 50% identical, more preferably at least 60% identical, and still more preferably 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide selected from the group consisting of the sequences of SEQ ID NO:2 and those encoded by the clone inserts of the deposited clone pool. By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid.

Further polypeptides of the present invention include polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. By a polypeptide having an amino acid sequence at least, for

example, 95% "similar" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is similar (i.e. contain identical or equivalent amino acid residues) to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% similar to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another non-equivalent amino acid.

These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. The query sequence may be an entire amino acid sequence selected from the group consisting of sequences of SEQ ID NO:2 and 4 those encoded by the clone inserts of the deposited clone pool or any fragment specified as described herein.

The variant polypeptides described herein are included in the present invention regardless of whether they have their normal biological activity. This is because even where a particular polypeptide molecule does not have biological activity, one of skill in the art would still know how to use the polypeptide, for instance, as a vaccine or to generate antibodies. Other uses of the polypeptides of the present invention that do not have FRADJ and/or CRYPTIC biological activity include, *inter alia,* as epitope tags, in epitope mapping, and as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods known to those of skill in the art. As described below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting FRADJ and/or CRYPTIC protein expression or as AGONISTS and ANTAGONISTS capable of enhancing or inhibiting FRADJ and/or CRYPTIC protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" FRADJ and/or CRYPTIC protein binding proteins, which are also candidate AGONISTS and ANTAGONISTS according to the present invention (*See, e.g.,* Fields *et al.* 1989), which disclosure is hereby incorporated by reference in its entirety.

Preparation of the Polypeptides of the Invention

The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. The polypeptides of the present invention are preferably provided in an isolated form, and may be partially or preferably substantially purified.

Consequently, the present invention also comprises methods of making the polypeptides of the invention, particularly polypeptides encoded by the cDNA of SEQ ID NO:1 or 3 or by the clone inserts

of the deposited clone pool, genomic DNA obtainable therefrom, or fragments thereof and methods of making the polypeptides of SEQ ID NO:2 or 4 fragments thereof. The methods comprise sequentially linking together amino acids to produce the nucleic polypeptides having the preceding sequences. In some embodiments, the polypeptides made by these methods are 150 amino acids or less in length. In other embodiments, the polypeptides made by these methods are 120 amino acids or less in length.

Isolation

From natural sources

The FRADJ and/or CRYPTIC proteins of the invention may be isolated from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured cells, of humans or non-human animals. Methods for extracting and purifying natural proteins are known in the art, and include the use of detergents or chaotropic agents to disrupt particles followed by differential extraction and separation of the polypeptides by ion exchange chromatography, affinity chromatography, sedimentation according to density, and gel electrophoresis. See, for example, "Methods in Enzymology, Academic Press, 1993" for a variety of methods for purifying proteins, which disclosure is hereby incorporated by reference in its entirety. Polypeptides of the invention also can be purified from natural sources using antibodies directed against the polypeptides of the invention, such as those described herein, in methods that are well known in the art of protein purification.

From recombinant sources

Preferably, the FRADJ and/or CRYPTIC polypeptides of the invention are recombinantly produced using routine expression methods known in the art. The polynucleotide encoding the desired polypeptide is operably linked to a promoter into an expression vector suitable for any convenient host. Both eukaryotic and prokaryotic host systems are used in forming recombinant polypeptides. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use.

Any FRADJ and/or CRYPTIC polynucleotide, including those described in SEQ ID NO:1 and 3, those of clone inserts of the deposited clone pool, and allelic variants thereof may be used to express FRADJ and/or CRYPTIC polypeptides. The nucleic acid encoding the FRADJ and/or CRYPTIC polypeptide to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The FRADJ and/or CRYPTIC insert in the expression vector may comprise the full coding sequence for the FRADJ and/or CRYPTIC protein or a portion thereof. For example, the FRADJ and/or CRYPTIC derived insert may encode a polypeptide comprising at least 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of a FRADJ and CRYPTIC protein selected

from the group consisting of sequences of SEQ ID NO:2 and 4, respectively, and polypeptides encoded by the clone inserts of the deposited clone pool.

Consequently, a further embodiment of the present invention is a method of making a polypeptide comprising a protein selected from the group consisting of sequences of SEQ ID NO:2 and 4 and polypeptides encoded by the clone inserts of the deposited clone pool, said method comprising the steps of

a) obtaining a cDNA comprising a sequence selected from the group consisting of: i) the sequences SEQ ID NO:1 or 3, ii) the sequences of clone inserts of the deposited clone pool one, iii) sequences encoding one of the polypeptide of SEQ ID NO:2 or 4, and iv) sequences of polynucleotides encoding a polypeptide which is encoded by one of the clone insert of the deposited clone pool;

b) inserting said cDNA in an expression vector such that the cDNA is operably linked to a promoter; and

c) introducing said expression vector into a host cell whereby said host cell produces said polypeptide.

In one aspect of this embodiment, the method further comprises the step of isolating the polypeptide. Another embodiment of the present invention is a polypeptide obtainable by the method described in the preceding paragraph.

The expression vector is any of the mammalian, yeast, insect or bacterial expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence is optimized for the particular expression organism in which the expression vector is introduced, as explained in U.S. Patent No. 5,082,767, which disclosure is hereby incorporated by reference in its entirety.

In one embodiment, the entire coding sequence of a FRADJ and/or CRYPTIC cDNA and the 3'UTR through the poly A signal of the cDNA is operably linked to a promoter in the expression vector. Alternatively, if the nucleic acid encoding a portion of the FRADJ and/or CRYPTIC protein lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. Similarly, if the insert from the FRADJ and/or CRYPTIC cDNA lacks a poly A signal, this sequence can be added to the construct by, for example, splicing out the Poly A signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the

LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex Thymidine Kinase promoter and the selectable neomycin gene. The nucleic acid encoding the FRADJ and/or CRYPTIC protein or a portion thereof is obtained by PCR from a vector containing a F RADJ and/or CRYPTIC cDNA selected from the group consisting of the sequences of SEQ ID NO:1 and 3 and the clone inserts of the deposited clone pool using oligonucleotide primers complementary to the FRADJ and/or CRYPTIC cDNA or portion thereof and containi ng restriction endonuclease sequences for Pst I incorporated into the 5' primer and BglII at the 5' end of the corresponding cDNA 3' primer, taking care to ensure that the sequence encoding the FRADJ and/or CRYPTIC protein or a portion thereof is positioned properly with respect to the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and dig ested with BglII.

In another embodiment, it is often advantageous to add to the recombinant polynucleotide additional nucleotide sequence which codes for secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

As a control, the expression vector lacking a cDNA insert is introduced into host cells or organisms.

Transfection of a FRADJ and/or CRYPTIC expressing vector into mouse NTH 3T3 cells is but one embodiment of introducing polynucleotides into host cells. Introduction of a polynucleotide encoding a polypeptide into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.* (1986), which disclosure is hereby incorporated by reference in its entirety. It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

Recombinant cell extracts, or proteins from the culture medium if the expressed polypeptide is secreted, are then prepared and proteins separated by gel electrophoresis. If desired, the proteins may be ammonium sulfate precipitated or separated based on size or charge prior to electrophoresis. The proteins present are detected using techniques such as Coomassie or silver staining or using antibodies against the protein encoded by the FRADJ and/or CRYPTIC cDNA of interest. Coomassie and silver staining techniques are familiar to those skilled in the art.

Proteins from the host cells or organisms containing an expression vector that contains the FRADJ and/or CRYPTIC cDNA or a fragment thereof are compared to those from the control cells or organism. The presence of a band from the cells containing the expression vector that is absent in control cells indicates that the FRADJ and/or CRYPTIC cDNA is expressed. Generally, the band corresponding

to the protein encoded by the FRADJ and/or CRYPTIC cDNA will have a mobility near that expected based on the number of amino acids in the open reading frame of the cDNA. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

Alternatively, the FRADJ and/or CRYPTIC polypeptide to be expressed may also be a product of transgenic animals, i.e., as a component of the milk of transgenic cows, goats, pigs or sheeps which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein of interest.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including differential extraction, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. See, for example, "Methods in Enzymology", *supra* for a variety of methods for purifying proteins. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. A recombinantly produced version of a FRADJ and/or CRYPTIC polypeptide can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson (1988), which disclosure is hereby incorporated by reference in its entirety. Polypeptides of the invention also can be purified from recombinant sources using antibodies directed against the polypeptides of the invention, such as those described herein, in methods which are well known in the art of protein purification.

Preferably, the recombinantly expressed FRADJ and/or CRYPTIC polypeptide is purified using standard immunochromatography techniques such as the one described in the section entitled "Immunoaffinity Chromatography". In such procedures, a solution containing the protein of interest, such as the culture medium or a cell extract, is applied to a column having antibodies against the protein attached to the chromatography matrix. The recombinant protein is allowed to bind the immunochromatography column. Thereafter, the column is washed to remove non-specifically bound proteins. The specifically bound secreted protein is then released from the column and recovered using standard techniques.

If antibody production is not possible, the FRADJ and/or CRYPTIC cDNA sequence or fragment thereof may be incorporated into expression vectors designed for use in purification schemes employing chimeric polypeptides. In such strategies the coding sequence of the FRADJ and/or CRYPTIC cDNA or fragment thereof is inserted in frame with the gene encoding the other half of the chimera. The other half of the chimera may be beta-globin or a nickel binding polypeptide encoding sequence. A chromatography matrix having antibody to beta-globin or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites may be engineered between the beta-globin gene or the

nickel binding polypeptide and the FRADJ and/or CRYPTIC cDNA or fragment thereof. Thus, the two polypeptides of the chimera may be separated from one another by protease digestion.

One useful expression vector for generating beta-globin chimerics is pSG5 (Stratagene), which encodes rabbit beta-globin. Intron II of the rabbit beta-globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis *et al.*, (1986) and many of the methods are available from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from the construct using *in vitro* translation systems such as the *In vitro* Express™ Translation Kit (Stratagene).

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

From chemical synthesis

In addition, polypeptides of the invention, especially short protein fragments, can be chemically synthesized using techniques known in the art (*See, e.g.*, Creighton, 1983; and Hunkapiller *et al.*, 1984), which disclosures are hereby incorporated by reference in their entireties. For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. A variety of methods of making polypeptides are known to those skilled in the art, including methods in which the carboxyl terminal amino acid is bound to polyvinyl benzene or another suitable resin. The amino acid to be added possesses blocking groups on its amino moiety and any side chain reactive groups so that only its carboxyl moiety can react. The carboxyl group is activated with carbodiimide or another activating agent and allowed to couple to the immobilized amino acid. After removal of the blocking group, the cycle is repeated to generate a polypeptide having the desired sequence. Alternatively, the methods described in U.S. Patent No. 5,049,656, which disclosure is hereby incorporated by reference in its entirety, may be used.

Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino

hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoroamino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Modifications

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques including, but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention that may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity. See U.S. Patent NO: 4,179,337. The chemical moieties for derivatization may be selected. See, U.S. Patent NO: 4,179,337, which disclosure is hereby incorporated by reference in its entirety. The chemical moieties for derivatization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, (coupling PEG to G-CSF), and Malik *et al.* (1992) (reporting pegylation of GM-CSF using tresyl chloride), which disclosures are hereby incorporated by reference in their entireties. For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Multimerization

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term "homomer", refers to a multimer containing only polypeptides corresponding to the amino acid sequences of SEQ ID NO:2 or 4 or encoded by the clone inserts of the deposited clone pool (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described

herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term "heteromer" refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences, which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925, which disclosure is hereby incorporated by reference in its entirety). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305,

the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins, and have since been found in a variety of different proteins (Landschulz *et al.*, 1988). Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe *et al.* (1994) and in U.S. patent application Ser. No. 08/446,922, which disclosure is hereby incorporated by reference in its entirety. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention. In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and

techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, 30 techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Mutated polypeptides

To improve or alter the characteristics of FRADJ and/or CRYPTIC polypeptides of the present invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions, or fusion proteins. Such modified polypeptides can show, e.g., increased/decreased biological activity or increased/decreased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Further, the polypeptides of the present invention may be produced as multimers including dimers, trimers and tetramers. Multimerization may be facilitated by linkers or recombinantly though heterologous polypeptides such as Fc regions.

N- and C-terminal deletions

It is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron *et al.* (1993), reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 N-terminal amino acid residues were missing. Accordingly, the present invention provides polypeptides having one or more residues deleted from the amino terminus of the polypeptides of SEQ ID NO:2 or 4 or that encoded by

the clone inserts of the deposited clone pool. Similarly, many examples of biologically functional C-terminal deletion mutants are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8 10 amino acid residues from the C-terminus of the protein (*See, e.g.,* Dobeli, *et al.* 1988), which disclosure is hereby incorporated by reference in its entirety. Accordingly, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of the polypeptides shown of SEQ ID NO:2 or 4 or encoded by the clone inserts of the deposited clone pool. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini as described below.

Other mutations

Other mutants in addition to N- and C-terminal deletion forms of the protein discussed above are included in the present invention. It also will be recognized by one of ordinary skill in the art that some amino acid sequences of the FRADJ and/or CRYPTIC polypeptides of the present invention can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the FRADJ and/or CRYPTIC polypeptides that show substantial FRADJ and/or CRYPTIC polypeptide activity. Such mutants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided.

There are two main approaches for studying the tolerance of an amino acid sequence to change (See, Bowie *et al.* 1994), which disclosure is hereby incorporated by reference in its entirety. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection.

The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality. These studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The studies indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described by Bowie *et al.* (supra) and the references cited therein.

Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Phe; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr. Thus, the fragment, derivative, analog, or homologue of the polypeptide of the present invention may be, for

example: (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or (ii) one in which one or more of the amino acid residues includes a substituent group; or (iii) one in which the FRADJ and/or CRYPTIC polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the pol ypeptide or a pro-protein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the FRADJ and/or CRYPTIC polypeptides of the present invention may include one or more amino acid substitutions, deletions, or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. The following groups of amino acids generally represent equivalent changes: (1) Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr; (2) Cys, Ser, Tyr, Thr; (3) Val, Ile, Leu, Met, Ala, Phe; (4) Lys, Arg, His; (5) Phe, Tyr, Trp, His.

A specific embodiment of a modified FRADJ and/or CRYPTIC peptide molecule of interest according to the present invention, includes, but is not limited to, a peptide molecule which is resistant to proteolysis, is a peptide in which the -CONH- peptide bond is modified and replaced by a (CH2NH) reduced bond, a (NHCO) retro inverso bond, a (CH2-O) methylene-oxy bond, a (CH2-S) thiomethylene bond, a (CH2CH2) carba bond, a (CO-CH2) cetomethylene bond, a (CHOH-CH2) hydroxyethylene bond), a (N-N) bound, a E-alcene bond or also a -CH=CH- bond. The invention also encompasses a huma FRADJ and/or CRYPTIC polypeptide or a fragment or a variant thereof in which at least one peptide bond has been modified as described above.

Amino acids in the FRADJ and/or CRYPTIC proteins of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (*See, e.g.*, Cunningham *et al.* 1989), which disclosure is hereby incorporated by reference in its entirety. The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity using assays appropriate for measuring the function of the particular protein. Of special interest are substitutions of charged amino acids with other charged or neutral amino acids that may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic, (*See, e.g.*, Pinckard *et al.*, 1967; Robbins, *et al.*, 1987; and Cleland, *et al.*, 1993).

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a FRADJ and/or CRYPTIC polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, not more than 40 conservative amino acid substitutions, not more than 30 conservative amino acid substitutions, and not more than 20 conservative amino acid substitutions. Also provided are polypeptides which comprise the amino acid sequence of a FRADJ and/or CRYPTIC polypeptide, having at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

Polypeptide fragments

Biologically active FRADJ and/or CRYPTIC polypeptides have a biological activity selected from the group consisting of: a reduction of the postprandial response of plasma free fatty acids, glucose, and/or triglycerides; increase in muscle free fatty acid oxidation *in vitro* and *ex vivo*; and sustained weight loss in patients, including those on a high fat/sucrose diet. Soluble forms of the FRADJ and/or CRYPTIC and membrane bound but biologically inactive FRADJ and/or CRYPTIC polypeptides are ANTAGONISTS of the natural membrane bound biologically active forms of FRADJ and/or CRYPTIC. FRADJ and/or CRYPTIC polypeptides are biologically inactive if, when membrane bound, do not elicit a biological activity described herein.

By "intact" or "full-length" FRADJ and/or CRYPTIC polypeptide as used herein is meant the full length polypeptide sequence of any FRADJ and/or CRYPTIC polypeptide, from the N-terminal methionine to the amino acid residue encoded by the codon preceeding the C-terminal stop codon. Examples of intact or full length FRADJ and CRYPTIC polypeptides are found in SEQ ID NO:2 and 4, respectively. The term "FRADJ and/or CRYPTIC polypeptide(s)" as used herein refers to the "intact" or "full-length" FRADJ and/or CRYPTIC polypeptide or fragments thereof. The FRADJ and/or CRYPTIC polypeptides may be limited to those having a particular activity, size, domain(s), etc. The term "fragment" means a polypeptide having a sequence that is entirely the same as part, but not all, of an intact or full-length FRADJ and/or CRYPTIC polypeptide. Such fragments may be "free-standing" (*i.e.* not part of or fused to other polypeptides), or one or more fragments may be present in a single polypeptide. FRADJ and/or CRYPTIC fragments are contiguous fragments of the full length FRADJ and/or CRYPTIC polypeptide unless otherwise specified.

The term "obesity-related activity" as used herein refers to at least one, and preferably all, of the activities described herein for FRADJ and/or CRYPTIC polypeptides. Assays for the determination of these activities are provided herein, and equivalent assays can be designed by those with ordinary skill in the art. Optionally, "obesity-related activity" can be selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity, or an activity within one of these categories. By "lipid partitioning" activity is meant the ability to effect the location of dietary lipids among the major tissue groups including, adipose tissue, liver, and muscle. By "lipid metabolism" activity is meant the

ability to influence the metabolism of lipids, e.g., through free fatty acid oxidation in skeletal muscle or reduction of the levels of triglycerides in the plasma and the muscle. By "insulin-like" activity is meant the ability of FRADJ and/or CRYPTIC polypeptides to modulate the levels of glucose in the plasma, preferably by not significantly increasing insulin levels, but by decreasing glucose levels similarly to the effects of insulin.

The biological activity of a compound of the present invention may be compared to the activity of another compound, e.g., wild type FRADJ and/or CRYPTIC or ligand. The term "significantly" as used herein is meant statistically significant as it is typically determined by those with ordinary skill in the art. For example, data are typically calculated as a mean ± sem, and a p-value < 0.05 is considered statistically significant. Statistical analysis is typically done using either the unpaired student's t test or the paired student's t test, as appropriate in each study. Examples of a significant change in ligand activity as a result of the presence a FRADJ and/or CRYPTIC AGONIST or ANTAGONIST of the invention compared to the absence of said AGONIST or ANTAGONIST include an increase or a decrease in a given parameter of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. One or more, but not necessarily all, of the measurable parameters will change significantly in the presence of said FRADJ and/or CRYPTIC AGONIST or ANTAGONIST as compared to the absence of said AGONIST or ANTAGONIST.

Representative "obesity-related assays" are provided in the Examples section and known in the art. These assays include, but are not limited to, methods of measuring the postprandial response, methods of measuring free fatty acid oxidation, and methods of measuring weight modulation. In preferred embodiments, the post-prandial response is measured in non-human animals, preferably mice. In preferred embodiments changes in dietary lipids are measured, preferably free fatty acids and/or triglycerides. In other embodiments, other physiologic parameters are measured including, but not limited to, levels of glucose, insulin, and leptin. In other preferred embodiments, free fatty acid oxidation is measured in cells *in vitro* or *ex vivo,* preferably in muscle cells or tissue of non-human animals, preferably mice. In yet other preferred embodiments weight modulation is measured in human or non-human animals, preferably rodents (rats or mice), primates, canines, felines or procines on a normal or high fat/sucrose diet. Optionally, "obesity-related activity" includes other activities not specifically identified herein. In general, "measurable parameters" relating to obesity and the field of metabolic research can be selected from the group consisting of free fatty acid levels, free fatty acid oxidation, triglyceride levels, glucose levels, insulin levels, leptin levels, food intake, weight, and leptin and lipoprotein binding.

In these obesity-related assays, preferred gene polypeptides of the invention, would cause a significant change in at least one of the measurable parameters selected from the group consisting of post-prandial lipemia, free fatty acid levels, triglyceride levels, glucose levels, free fatty acid oxidation, and weight. Alternatively, preferred gene polypeptides of the invention, would have a significant change

in leptin activity and an in lipoprotein activity. By "LSR" activity is meant expression of lipolysis stimulated receptor (LSR) on the surface of the cell, or in a particular conformation, as well as its ability to bind, uptake, and degrade leptin and lipoprotein. By "leptin" activity is meant its binding, uptake and degradation by LSR, as well as its transport across a blood brain barrier, and potentially these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Similarly, by "lipoprotein" activity is meant its binding, uptake and degradation by LSR, as well as these occurrences where LSR is not necessarily the mediating factor or the only mediating factor.

The invention is drawn, *inter alia*, to isolated, purified or recombinant FRADJ and/or CRYPTIC polypeptides. FRADJ and/or CRYPTIC polypeptides of the invention are useful for reducing (AGONISTS of FRADJ and/or CRYPTIC polypeptides) or increasing (ANTAGONISTS of FRADJ and/or CRYPTIC polypeptides) body weight either as a cosmetic treatment or for treatment or prevention of diseases and disorders as discussed or described herein. FRADJ and/or CRYPTIC polypeptides are also useful *inter alia* in screening assays for AGONISTS or ANTAGONISTS of gene activity, for raising gene-specific antibodies, and in diagnostic assays. When used for cosmetic treatments, or for the treatment or prevention of diseases, disorders, or conditions, one or more gene polypeptides can be provided to a subject. Thus, various fragments of the full-length protein can be combined into a "cocktail" for use in the various treatment regimens.

The full-length FRADJ polypeptide is comprised of at least 3 distinct regions including:

1. an N-terminal putative signal peptide comprising amino acids from about amino acids 1-27 of SEQ ID NO:2;
2. an extracellular domain comprising a LIGAND binding portion (and Cys-rich regions) and comprising amino acids from about amino acids 28-78 of SEQ ID NO:2;
3. a transmembrane domain comprising amino acids from about amino acids 79-101 of SEQ ID NO:2; and
4. an intracellular domain comprising amino acids from about amino acids 102-129 of SEQ ID NO:2.

The full-length CRYPTIC polypeptide is comprised of at least 3 distinct regions including:

1. an N-terminal putative signal peptide comprising amino acids from about amino acids 1-27 of SEQ ID NO:4;
2. an extracellular domain comprising a LIGAND binding portion (and Cys-rich regions) and comprising amino acids from about amino acids 28-43 of SEQ ID NO:4;

3. a transmembrane domain comprising amino acids from about amino acids 44-66 of SEQ ID NO: 4; and

4. an intracellular domain comprising amino acids from about amino acids 67-94 of SEQ ID NO:4.

The FRADJ and/or CRYPTIC polypeptides of the present invention are preferably provided in an isolated form, and may be partially or substantially purified. A recombinantly produced version of a FRADJ and/or CRYPTIC polypeptide fragment can be substantially purified by the one-step method described by Smith et al. ((1988) Gene 67(1):31-40) or by the methods described herein or known in the art (see, e.g., Examples 1-3). Fragments of the invention also can be purified from natural or recombinant sources using antibodies directed against the polypeptide fragments of the invention by methods known in the art of protein purification.

Preparations of FRADJ and/or CRYPTIC polypeptides of the invention involving a partial purification of or selection for the FRADJ and/or CRYPTIC polypeptides are also specifically contemplated. These crude preparations are envisioned to be the result of the concentration of cells expressing FRADJ and/or CRYPTIC polypeptides with perhaps a few additional purification steps, but prior to complete purification of the fragment. The cells expressing FRADJ and/or CRYPTIC polypeptides are present in a pellet, they are lysed, or the crude polypeptide is lyophilized, for example.

FRADJ and/or CRYPTIC polypeptides can be any integer in length from at least 6 consecutive amino acids to the length of a full length FRADJ and/or CRYPTIC polypeptide as disclosed in the sequence listing. Thus, for human FRADJ and CRYPTIC (SEQ ID NO:2 and 4, respectively), a FRADJ and/or CRYPTIC polypeptide can be any one integer of consecutive amino acids from 6 to 129, for example. The term "integer" is used herein in its mathematical sense and thus representative integers include: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129.

Each FRADJ and/or CRYPTIC polypeptide as described herein can be further specified in terms of its N-terminal and C-terminal positions. For example, every combination of a N-terminal and C-terminal position that fragments of from 6 contiguous amino acids to 1 amino acids less than the full length FRADJ and/or CRYPTIC polypeptide could occupy, on any given intact and contiguous full length FRADJ and/or CRYPTIC polypeptide sequence are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, 15-20, 16-21, 17-22, 18-23, 19-

24, 20-25, 21-26, 22-27, 23-28, 24-29, 25-30, 26-31, 27-32, 28-33, 29-34, 30-35, 31-36, 32-37, 33-38, 34-39, 35-40, 36-41, 37-42, 38-43, 39-44, 40-45, 41-46, 42-47, 43-48, 44-49, 45-50, 46-51, 47-52, 48-53, 49-54, 50-55, 51-56, 52-57, 53-58, 54-59, 55-60, 56-61, 57-62, 58-63, 59-64, 60-65, 61-66, 62-67, 63-68, 64-69, 65-70, 66-71, 67-72, 68-73, 69-74, 70-75, 71-76, 72-77, 73-78, 74-79, 75-80, 76-81, 77-82, 78-83, 79-84, 80-85, 81-86, 82-87, 83-88, 84-89, 85-90, 86-91, 87-92, 88-93, 89-94, 90-95, 91-96, 92-97, 93-98, 94-99, 95-100, 96-101, 97-102, 98-103, 99-104, 100-105, 101-106, 102-107, 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116, 112-117, 113-118, 114-119, 115-120, 116-121, 117-122, 118-123, 119-124, 120-125, 121-126, 122-127, 123-128, 124-129 of SEQ ID NO:2 or 4. A 125 consecutive amino acid fragment could occupy positions selected from the group consisting of 1 -125, 2-126, 3-127, 4-128, and 5-129 of SEQ ID NO:2 or 4, respectively. Similarly, the positions occupied by all the other fragments of sizes between 6 amino acids and 129 amino acids on SEQ ID NO:2 or 4, respectively are included in the present invention and can also be immediately envisaged based on these two examples and therefore, are not individually listed solely for the purpose of not unnecessarily lengthening the specification. In addition, the positions occupied by fragments of 6 consecutive amino acids to 1 amino acid less than any other full length FRADJ and/or CRYPTIC polypeptide can also be envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification.

The FRADJ and/or CRYPTIC polypeptides of the present invention may alternatively be described by the formula "n to c" (inclusive); where "n" equals the N-terminal most amino acid position (as defined by the sequence listing) and "c" equals the C-terminal most amino acid position (as defined by the sequence listing) of the polypeptide defined by the formula; and further where "n" equals an integer between 1 and the number of amino acids of the full length polypeptide sequence of the present invention minus 5 (for SEQ ID NO:2 or 4, respectively); and where "c" equals an integer between 6 and the number of amino acids of the full length polypeptide sequence (for SEQ ID NO:2 or 4, respectively); and where "n" is an integer smaller then "c" by at least 5. Therefore, for SEQ ID NO:2 or 4, "n" is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124; and "c" is any integer selected from the group consisting of: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129. Every combination of "n" and "c" positions are included as

specific embodiments or species of the invention. Moreover, the formula "n" to "c" may be modified as "'n1 – n2" to "c1 – c2"', wherein "n1 – n2" and "c1 – c2" represent positional ranges selected from any two integers above which represent amino acid positions of the sequence listing. Alternative formulas include "'n1 – n2" to "c"' and "'n" to "c1 – c2"'.

It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise.

The present invention also provides for the exclusion of any individual fragment species specified by N-terminal and C-terminal positions or of any sub-genus of fragments specified by size in amino acid residues as described above.

Further, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may make up a polypeptide in any combination and may optionally include non-FRADJ and/or CRYPTIC polypeptide sequence as well.

FRADJ and/or CRYPTIC polypeptides of the invention include variants, fragments, analogs and derivatives of the FRADJ and/or CRYPTIC polypeptides described above, including modified FRADJ and/or CRYPTIC polypeptides.

Epitopes and Antibody Fusions:

A preferred embodiment of the present invention is directed to epitope-bearing polypeptides and epitope-bearing polypeptide fragments. These epitopes may be "antigenic epitopes" or both an "antigenic epitope" and an "immunogenic epitope". An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response *in vivo* when the polypeptide is the immunogen. On the other hand, a region of polypeptide to which an antibody binds is defined as an "antigenic determinant" or "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*See, e.g.,* Geysen, *et al.*, 1984), which disclosure is hereby incorporated by reference in its entirety. It is particularly noted that although a particular epitope may not be immunogenic, it is nonetheless useful since antibodies can be made to both immunogenic and an tigenic epitopes.

An epitope can comprise as few as 3 amino acids in a spatial conformation, which is unique to the epitope. Generally an epitope consists of at least 6 such amino acids, and more often at least 8 - 10 such amino acids. In preferred embodiment, antigenic epitopes comprise a number of amino acids that is any integer between 3 and 50. Fragments which function as epitopes may be produced by any conventional means (*See, e.g.,* Houghten, 1985), also further described in U.S. Patent No. 4,631,21, which disclosures are hereby incorporated by reference in their entireties. Methods for determining the amino acids which make up an epitope include x-ray crystallography, 2-dimensional nuclear magnetic resonance, and epitope mapping, e.g., the Pepscan method described by Geysen *et al.* (1984); PCT

Publication No. WO 84/03564; and PCT Publication No. WO 84/03506, which disclosures are hereby incorporated by reference in their entireties. Another example is the algorithm of Jameson and Wolf, (1988) (said reference incorporated by reference in its entirety). The Jameson-Wolf antigenic analysis, for example, may be performed using the computer program PROTEAN, using default parameters (Version 4.0 Windows, DNASTAR, Inc., 1228 South Park Street Madison, WI.

Antigenic epitopes are predicted by the Jameson-Wolf algorithm for the polypeptides of SEQ ID NO:2 or 4. For each FRADJ and/or CRYPTIC polypeptide referred to by its sequence identification number in the first column, a list of antigenic epitopes is given in the second column, each epitope being separated by a coma. Polypeptides of the present invention that are not specifically described as immunogenic are not considered non-antigenic. This is because they may still be antigenic *in vivo* but merely not recognized as such by the particular algorithm used. Alternatively, the polypeptides are most likely antigenic *in vitro* using methods such a phage display. All fragments of the polypeptides of the present invention, at least 6 amino acids residues in length, are included in the present invention as being useful as antigenic epitope. Amino acid residues comprising other immunogenic epitopes may be determined by algorithms similar to the Jameson-Wolf analysis or by *in vivo* testing for an antigenic response using the methods described herein or those known in the art.

Therefore, the present invention encompasses isolated, purified, or recombinant polypeptides which consist of, consist essentially of, or comprise a contiguous span of at least 6, preferably at least 8 to 10, more preferably 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, or 125 amino acids of a sequence selected from the group consisting of the sequences of SEQ ID NO:2 or 4, respectively, to the extent that a contiguous span of these lengths is consistent with the lengths of said selected sequence, where said contiguous span comprises at least 1, 2, 3, 5, or 10 amino acids positions of an epitope of said selected sequence. The present invention also encompasses isolated, purified, or recombinant polypeptides comprising, consisting essentially of, or consisting of a contiguous span of at least 6, preferably at least 8 to 10, more preferably 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, or 125 amino acids of a sequence selected from the group consisting of the sequences of SEQ ID NO:2 or 4, respectively, to the extent that a contiguous span of these lengths is consistent with the lengths of said selected sequence, where said contiguous span is an epitope of said selected sequence. The present invention also encompasses isolated, purified, or recombinant polypeptides that comprise, consist of or consist essentially of an epitope of a sequence selected from the group consisting of the sequences of SEQ ID NO:2 or 4, respectively.

The epitope-bearing fragments of the present invention preferably comprises 6 to 50 amino acids (i.e. any integer between 6 and 50, inclusive) of a polypeptide of the present invention. Also, included in the present invention are antigenic fragments between the integers of 6 and the full length FRADJ and/or CRYPTIC sequence of the sequence listing. All combinations of sequences between the integers of 6 and the full-length sequence of a FRADJ and/or CRYPTIC polypeptide are included. The epitope-

bearing fragments may be specified by either the number of contiguous amino acid residues (as a sub-genus) or by specific N-terminal and C-terminal positions (as species) as described above for the polypeptide fragments of the present invention. Any number of epitope-bearing fragments of the present invention may also be excluded in the same manner.

Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies that specifically bind the epitope (See, Wilson *et al.*, 1984; and Sutcliffe, *et al.*, 1983), which disclosures are hereby incorporated by reference in their entireties. The antibodies are then used in various techniques such as diagnostic and tissue/cell identification techniques, as described herein, and in purification methods such as immunoaffinity chromatography.

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (See, Sutcliffe *et al.*, supra; Wilson *et al.*, supra; Chow *et al.*;(1985) and Bittle, *et al.*, (1985), which disclosures are hereby incorporated by reference in their entireties). A preferred immunogenic epitope includes the natural FRADJ and/or CRYPTIC protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rab bit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.).

Epitope-bearing polypeptides of the present invention are used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods (*See, e.g.,* Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*, and Bittle, *et al.*, *supra*). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as -maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µgs of peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody, which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, any combination thereof including both entire domains and portions thereof) resulting in chimeric polypeptides. These fusion proteins facilitate purification, and show an increased half-life *in vivo*. This has been shown, *e.g.*, for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (*See, e.g.*, EPA 0,394,827; and Traunecker *et al.*, 1988), which disclosures are hereby incorporated by reference in their entireties. Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion can also be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone (*See, e.g.*, Fountoulakis *et al.*, 1995), which disclosure is hereby incorporated by reference in its entirety. Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag to aid in detection and purification of the expressed polypeptide.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the present invention thereby effectively generating AGONISTS and ANTAGONISTS of the polypeptides. See, for example, U.S. Patent Nos.: 5,605,793; 5,811,238; 5,834,252; 5,837,458; and Patten, *et al.*, (1997); Harayama, (1998); Hansson, *et al* (1999); and Lorenzo and Blasco, (1998). (Each of these documents are hereby incorporated by reference). In one embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of coding polynucleotides of the invention, or the polypeptides encoded thereby may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

The present invention further encompasses any combination of the polypeptide fragments listed in this section.

Antibodies:

Definitions

The present invention further relates to antibodies and T-cell antigen receptors (TCR), which specifically bind the polypeptides, and more specifically, the epitopes of the polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. The term "antibody" (Ab) refers to a polypeptide or group of polypeptides which are comprised of at least one binding domain, where a binding domain is formed from the folding of variable domains of an antibody molecule to form three-

dimensional binding spaces with an internal surface shape and charge distribution complementary to th e features of an antigenic determinant of an antigen, which allows an immunological reaction with the antigen. As used herein, the term "antibody" is meant to include whole antibodies, including single-chain whole antibodies, and antigen binding fragments thereof. In a preferred embodiment the antibodies are human antigen binding antibody fragments of the present invention include, but are not limited to, Fab, Fab' F(ab)2 and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of var iable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes chimeric, humanized, and human monoclonal and polyclonal antibodies, which specifically bind the polypeptides of the present invention. The present invent ion further includes antibodies that are anti-idiotypic to the antibodies of the present invention.

The antibodies of the present invention may be monospecific, bispecific, and trispecific or have greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.* (1991); US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny *et al.* (1992), which disclosures are hereby incorporated by reference in their entireties.

Antibodies of the present invention may be described or specified in terms of the epitope(s) or epitope-bearing portion(s) of a polypeptide of the present invention, which are recognized or specifically bound by the antibody. The antibodies may specifically bind a complete protein encoded by a nucleic acid of the present invention, or a fragment thereof. Therefore, the epitope(s) or epitope bearing polypeptide portion(s) may be specified as described herein, *e.g.*, by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or otherwise described herein (including the sequence listing). Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded as individual species. Therefore, the present invention i ncludes antibodies that specifically bind specified polypeptides of the present invention, and allows for the exclusion of the same.

Thus, another embodiment of the present invention is a purified or isolated antibody capable of specifically binding to a polypeptide comprising a sequence selected from the group consisting of the sequences of SEQ ID NO:2 or 4 and the sequences of the clone inserts of the deposited clone pool. In

one aspect of this embodiment, the antibody is capable of binding to an epitope-containing polypeptide comprising at least 6 consecutive amino acids, preferably at least 8 to 10 consecutive amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 consecutive amino acids of a sequence selected from the group consisting of SEQ ID NO:2 or 4, respectively and sequences of the clone inserts of the deposited clone pool.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not specifically bind any other analog, ortholog, or homologue of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein, e.g., using FASTDB and the parameters set forth herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies, which only bind polypeptides encoded by polynucleotides, which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or Kd less than $5X10^{-6}M$, $10^{-6}M$, $5X10^{-7}M$, $10^{-7}M$, $5X10^{-8}M$, $10^{-8}M$, $5X10^{-9}M$, $10^{-9}M$, $5X10^{-10}M$, $10^{-10}M$, $5X10^{-11}M$, $10^{-11}M$, $5X10^{-12}M$, $10^{-12}M$, $5X10^{-13}M$, $10^{-13}M$, $5X10^{-14}M$, $10^{-14}M$, $5X10^{-15}M$, and $10^{-15}M$.

The invention also concerns a purified or isolated antibody capable of specifically binding to a mutated FRADJ and/or CRYPTIC protein or to a fragment or variant thereof comprising an epitope of the mutated FRADJ and/or CRYPTIC protein.

Preparation of antibodies

The antibodies of the present invention may be prepared by any suitable method known in the art. Some of these methods are described in more detail in the example entitled "Preparation of Antibody Compositions to the FRADJ and/or CRYPTIC protein". For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing "polyclonal antibodies". As used herein, the term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology but it rather refers to an antibody that is derived from a single clone, including eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technology.

Hybridoma techniques include those known in the art (*See, e.g.*, Harlow *et al.* 1988; Hammerling, *et al*, 1981). (Said references incorporated by reference in their entireties.) Fab and F(ab')2 fragments may be produced, for example, from hybridoma-produced antibodies by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle, which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman *et al.* (1995); Ames, *et al.* (1995); Kettleborough, *et al.* (1994); Persic, *et al.* (1997); Burton *et al.* (1994); PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516, 637, 5,780,225, 5,658,727 and 5,733,743 (said references incorporated by reference in their entireties).

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' F(ab)2 and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.* (1992); and Sawai *et al.* (1995); and Better *et al.* (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al.* (1991); Shu *et al.* (1993); and Skerra *et al.* (1988), which disclosures are hereby incorporated by reference in their entireties. For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, (1985); Oi *et al.*, (1986); Gillies *et al.* (1989); and US Patent 5,807,715, which disclosures are hereby incorporated by reference in their entireties. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent 5,530,101; and 5,585,089), veneering or resurfacing, (EP 0 592 106; EP 0 519 596; Padlan, 1991; Studnicka *et al.*, 1994; Roguska *et al.*, 1994), and chain shuffling (US Patent 5,565,332), which disclosures are hereby incorporated by reference in their entireties. Human antibodies can be made by a variety of methods known in the art including phage display methods described above. *See also*, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; WO 98/46645; WO 98/50433; WO 98/24893;

WO 96/34096; WO 96/33735; and WO 91/10741 (said references incorporated by reference in their entireties).

Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., WO 92/08495; WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387, which disclosures are hereby incorporated by reference in their entireties. Fused antibodies may also be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *vitro* immunoassays and purification methods using methods known in the art (*See e.g.*, Harbor *et al. supra*; WO 93/21232; EP 0 439 095; Naramura, M. *et al.* 1994; US Patent 5,474,981; Gillies *et al.*, 1992; Fell *et al.*, 1991) (said references incorporated by reference in their entireties).

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half-life of the polypeptides or for use in immunoassays using methods known in the art. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. *See e.g.*, US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi *et al.* (1991); Zheng *et al.* (1995); and Vil *et al.* (1992) (said references incorporated by reference in their entireties).

Non-human animals or mammals, whether wild-type or transgenic, which express a different species of FRADJ and/or CRYPTIC than the one to which antibody binding is desired, and animals which do not express FRADJ and/or CRYPTIC (i.e. a FRADJ and/or CRYPTIC knock out animal as described herein) are particularly useful for preparing antibodies. FRADJ and/or CRYPTIC knock out animals will recognize all or most of the exposed regions of a FRADJ and/or CRYPTIC protein as foreign antigens, and therefore produce antibodies with a wider array of FRADJ and/or CRYPTIC

epitopes. Moreover, smaller polypeptides with only 10 to 30 amino acids may be useful in obtaining specific binding to any one of the FRADJ and/or CRYPTIC proteins. In addition, the humoral immune system of animals that produce a species of FRADJ and/or CRYPTIC that resembles the antigenic sequence will preferentially recognize the differences between the animal's native FRADJ and/or CRYPTIC species and the antigen sequence, and produce antibodies to these unique sites in the antigen sequence. Such a technique will be particularly useful in obtaining antibodies that specifically bind to any one of the FRADJ and/or CRYPTIC proteins.

The antibodies of the invention may be labeled by any one of the radioactive, fluorescent or enzymatic labels known in the art.

Uses of Polynucleotides

To find similar sequences

Polynucleotides of the invention may be used to isolate and/or purify nucleic acids similar thereto using any methods well known to those skilled in the art including the techniques based on hybridization or on amplification described in this section. These methods may be used to obtain the genomic DNAs which encode the mRNAs from which the FRADJ and/or CRYPTIC cDNA are derived, mRNAs corresponding to FRADJ and/or CRYPTIC cDNA, or nucleic acids which are homologous to FRADJ and/or CRYPTIC cDNA or fragments thereof, such as variants, species homologues or or thologs. Thus, a plurality of cDNA similar to FRADJ and/or CRYPTIC polynucleotides may be provided as cDNA libraries for subsequent evaluation of the encoded proteins or use in diagnostic assays as described herein. cDNA prepared by any method described therein may be subsequently engineered to obtain nucleic acids which include desired fragments of the cDNA using conventional techniques such as subcloning, PCR, or *in vitro* oligonucleotide synthesis. For example, nucleic acids that include only the coding sequences may be obtained using techniques known to those skilled in the art. Similarly, nucleic acids containing any other desired fragment of the coding sequences for the encoded protein may be obtained.

Indeed, cDNA of the present invention or fragments thereof may be used to isolate nucleic acids similar to cDNA from a cDNA library or a genomic DNA library. Such cDNA libraries or genomic DNA libraries may be obtained from a commercial source or made using techniques familiar to those skilled in the art such as those described in PCT publication WO 00/37491, which disclosure is hereby incorporated by reference in its entirety. Examples of methods for obtaining nucleic acids similar to FRADJ and/or CRYPTIC polynucleotides are described below.

Hybridization-based methods

Techniques for identifying cDNA clones in a cDNA library which hybridize to a given probe sequence are disclosed in Sambrook *et al.*, (1989) and in Hames and Higgins (1985), the disclosures of

which are incorporated herein by reference in their entireties. The same techniques may be used to isolate genomic DNAs.

Briefly, cDNA or genomic DNA clones that hybridize to the detectable probe are identified and isolated for further manipulation as follows. Any polynucleotide fragment of the invention may be used as a probe, in particular those defined in "Polynucleotide Fragments" can be used in a variety of ways, including, but not limited to, expressing the polypeptide in recombinant cells for use in screening assays for ANTAGONISTS and AGONISTS of its activity as well as to facilitate its purification for use in a variety of ways including, but not limited to screening assays for AGONISTS and ANTAGONISTS of its activity, diagnostic screens, and raising antibodies, as well as treatment and/or prevention of diseases and disorders disclosed herein.

The invention relates to the polynucleotides encoding gene polypeptides and variant polypeptide fragments thereof as described herein. These polynucleotides may be purified, isolated, and/or recombinant. Preferred gene polynucleotides of the invention are those that encode gene polypeptides of the invention have a biological activity as described and discussed herein.

Identification of genes associated with hereditary diseases or drug response

This example illustrates an approach useful for the association of FRADJ and/or CRYPTIC cDNA or genomic DNAs with particular phenotypic characteristics. In this example, a particular FRADJ and/or CRYPTIC cDNA or genomic DNA is used as a test probe to associate that FRADJ and/or CRYPTIC cDNA or genomic DNA with a particular phenotypic characteristic.

FRADJ and/or CRYPTIC cDNA or genomic DNAs are mapped to a particular location on a human chromosome using techniques such as those described herein or other techniques known in the art. A search of Mendelian Inheritance in Man (V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) reveals the region of the human chromosome which contains the FRADJ and/or CRYPTIC cDNA or genomic DNA to be a very gene rich region containing several known genes and several diseases or phenotypes for which genes have not been identified. The gene corresponding to this FRADJ and/or CRYPTIC cDNA or genomic DNA thus becomes an immediate candidate for each of these genetic diseases.

Cells from patients with these diseases or phenotypes are isolated and expanded in culture. PCR primers from the FRADJ and/or CRYPTIC cDNA or genomic DNA are used to screen genomic DNA, mRNA or cDNA obtained from the patients. FRADJ and/or CRYPTIC cDNA or genomic DNAs that are not amplified in the patients can be positively associated with a particular disease by further analysis. Alternatively, the PCR analysis may yield fragments of different lengths when the samples are derived from an individual having the phenotype associated with the disease than when the sample is derived from a healthy individual, indicating that the gene containing the cDNA may be responsible for the genetic disease.

Uses of polynucleotides in recombinant vectors

The present invention also relates to recombinant vectors, which include the isolated polynucleotides of the present invention, and to host cells recombinant for a polynucleotide of the invention, such as the above vectors, as well as to methods of making such vectors and host cells and for using them for production of FRADJ and/or CRYPTIC polypeptides by recombinant techniques.

Recombinant Vectors

The term "vector" is used herein to designate either a circular or a linear DNA or RNA molecule, which is either double-stranded or single-stranded, and which comprise at least one polynucleotide of interest that is sought to be transferred in a cell host or in a unicellular or multicellular host organism. The present invention encompasses a family of recombinant vectors that comprise a regulatory polynucleotide and/or a coding polynucleotide derived from either the FRADJ and/or CRYPTIC genomic sequence or the cDNA sequence. Generally, a recombinant vector of the invention may comprise any of the polynucleotides described herein, including regulatory sequences, coding sequences and polynucleotide constructs, as well as any FRADJ and/or CRYPTIC primer or probe as defined herein.

In a first preferred embodiment, a recombinant vector of the invention is used to amplify the inserted polynucleotide derived from a FRADJ and/or CRYPTIC genomic sequence or a FRADJ and/or CRYPTIC cDNA, for example any cDNA selected from the group consisting of sequences of SEQ ID NO:1 or 3, sequences of clone inserts of the deposited clone pool, variants and fragments thereof in a suitable cell host, this polynucleotide being amplified at every time that the recombinant vector replicates.

A second preferred embodiment of the recombinant vectors according to the invention comprises expression vectors comprising either a regulatory polynucleotide or a coding nucleic acid of the invention, or both. Within certain embodiments, expression vectors are employed to express a FRADJ and/or CRYPTIC polypeptide that can be then purified and, for example be used in LIGAND screening assays or as an immunogen in order to raise specific antibodies directed against the FRADJ and/or CRYPTIC protein. In other embodiments, the expression vectors are used for constructing transgenic animals and also for gene therapy. Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements, such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in host cells. Dominant drug selection markers for establishing permanent, stable cell clones expressing the products are generally included in the expression vectors of the invention, as they are elements that link expression of the drug selection markers to expression of the polypeptide.

More particularly, the present invention relates to expression vectors which include nucleic acids encoding a FRADJ and/or CRYPTIC protein, preferably a FRADJ and/or CRYPTIC protein with an amino acid sequence selected from the group consisting of sequences of SEQ ID NO:2 or 4, sequences of

polypeptides encoded by the clone inserts of the deposited clone pool, variants and fragments thereof. The polynucleotides of the present invention may be used to express an encoded protein in a host organism to produce a beneficial effect. In such procedures, the encoded protein may be transiently expressed in the host organism or stably expressed in the host organism. The encoded protein may have any of the activities described herein. The encoded protein may be a protein that the host organism lacks or, alternatively, the encoded protein may augment the existing levels of the protein in the host organism.

Some of the elements that can be found in the vectors of the present invention are described in further detail in the following sections.

General features of the expression vectors of the invention

A recombinant vector according to the invention comprises, but is not limited to, a YAC (Yeast Artificial Chromosome), a BAC (Bacterial Artificial Chromosome), a phage, a phagemid, a cosmid, a plasmid or even a linear DNA molecule that may comprise a chromosomal, non-chromosomal, semi-synthetic and synthetic DNA. Such a recombinant vector can comprise a transcriptional unit comprising an assembly of:

(1) a genetic element or elements having a regulatory role in gene expression, for example promoters or enhancers. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp in length that act on the promoter to increase the transcription.

(2) a structural or coding sequence which is transcribed into mRNA and eventually translated into a polypeptide, said structural or coding sequence being operably linked to the regulatory elements described in (1); and

(3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, when a recombinant protein is expressed without a leader or transport sequence, it may include a N-terminal residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

Generally, recombinant expression vectors will include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably a leader sequence capable of directing secretion of the translated protein into the periplasmic space or the extracellular medium. In a specific embodiment wherein the vector is adapted for transfecting and expressing desired sequences in mammalian host cells, preferred vectors will comprise an origin of replication in the desired host, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation

signal, splice donor and acceptor sites, transcriptional termination sequences, and 5'-flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example SV40 origin, early promoter, enhancer, splice and polyadenylation signals may be used to provide the required non-transcribed genetic elements.

The *in vivo* expression of a FRADJ and/or CRYPTIC polypeptide of the present invention may be useful in order to correct a genetic defect related to the expression of the native gene in a host organism or to the production of a biologically inactive FRADJ and/or CRYPTIC protein. Consequently, the present invention also comprises recombinant expression vectors mainly designed for the *in vivo* production of a FRADJ and/or CRYPTIC polypeptide the present invention by the introduction of the appropriate genetic material in the organism or the patient to be treated. This genetic material may be introduced *in vitro* in a cell that has been previously extracted from the organism, the modified cell being subsequently reintroduced in the said organism, directly *in vivo* into the appropriate tissue.

Regulatory Elements

The suitable promoter regions used in the expression vectors according to the present invention are chosen taking into account the cell host in which the heterologous gene has to be expressed. The particular promoter employed to control the expression of a nucleic acid sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell, such as, for example, a human or a viral promoter.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed. Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Promoter regions can be selected from any desired gene using, for example, CAT (chloramphenicol transferase) vectors and more preferably pKK232-8 and pCM7 vectors.

Preferred bacterial promoters are the LacI, LacZ, the T3 or T7 bacteriophage RNA polymerase promoters, the gpt, lambda PR, PL and trp promoters (EP 0036776), the polyhedrin promoter, or the p10 protein promoter from baculovirus (Kit Novagen), (Smith *et al.*, 1983; O'Reilly *et al.*, 1992), which disclosures are hereby incorporated by reference in their entireties, the lambda PR promoter or also the trc promoter.

Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-L. Selection of a convenient vector and promoter is well within the level of ordinary skill in the art. The choice of a promoter is well within the

ability of a person skilled in the field of genetic engineering. For example, one may refer to the book of Sambrook *et al.*, (1989) or also to the procedures described by Fuller *et al.*, (1996), which disclosures are hereby incorporated by reference in their entireties.

Other regulatory elements

Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed such as human growth hormone and SV40 polyadenylation sig nals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

Selectable Markers

Selectable markers confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. The selectable marker genes for selection of transformed host cells are preferably dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for *S. cerevisiae* or tetracycline, rifampicin or ampicillin resistance in *E. Coli*, or levan saccharase for mycobacteria, this latter marker being a negative selection marker.

Preferred Vectors

Bacterial vectors

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and a bacterial origin of replication derived from commercially available plasmids comprising genetic elements of pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia, Uppsala, Sweden), and pGEM1 (Promega Biotec, Madison, WI, USA).

Large numbers of other suitable vectors are known to those of skill in the art, and commercially available, such as the following bacterial vectors: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16A, pNH18A, pNH46A (StrataFRADJ and/or CRYPTIC); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pQE-30 (QIAexpress).

Bacteriophage vectors

The P1 bacteriophage vector may contain large inserts ranging from about 80 to about 100 kb. The construction of P1 bacteriophage vectors such as p158 or p158/neo8 are notably described by Sternberg (1992, 1994), which disclosure is hereby incorporated by reference in its entirety. Recombinant P1 clones comprising FRADJ and/or CRYPTIC nucleotide sequences may be designed for

inserting large polynucleotides of more than 40 kb (See Linton *et al.*, 1993), which disclosure is hereby incorporated by reference in its entirety. To generate P1 DNA for transgenic experiments, a preferred protocol is the protocol described by McCormick *et al.* (1994), which disclosure is hereby incorporated by reference in its entirety. Briefly, *E. coli* (preferably strain NS3529) harboring the P1 plasmid are grown overnight in a suitable broth medium containing 25 µg/ml of kanamycin. The P1 DNA is prepared from the *E. coli* by alkaline lysis using the Qiagen Plasmid Maxi kit (Qiagen, Chatsworth, CA, USA), according to the manufacturer's instructions. The P1 DNA is purified from the bacterial lysate on two Qiagen-tip 500 columns, using the washing and elution buffers contained in the kit. A phenol/chloroform extraction is then performed before precipitating the DNA with 70% ethanol. After solubilizing the DNA in TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA), the concentration of the DNA is assessed by spectrophotometry.

When the goal is to express a P1 clone comprising FRADJ and/or CRYPTIC nucleotide sequences in a transgenic animal, typically in transgenic mice, it is desirable to remove vector sequences from the P1 DNA fragment, for example by cleaving the P1 DNA at rare-cutting sites within the P1 polylinker (*Sfi*I, *Not*I or *Sal*I). The P1 insert is then purified from vector sequences on a pulsed-field agarose gel, using methods similar to those originally reported for the isolation of DNA from YACs (*See e. g.*, Schedl *et al.*, 1993a; Peterson *et al.*, 1993), which disclosures are hereby incorporated by reference in their entireties. At this stage, the resulting purified insert DNA can be concentrated, if necessary, on a Millipore Ultrafree-MC Filter Unit (Millipore, Bedford, MA, USA – 30,000 molecular weight limit) and then dialyzed against microinjection buffer (10 mM Tris-HCl, pH 7.4; 250 µM EDTA) containing 100 mM NaCl, 30 µM spermine, 70 µM spermidine on a microdyalisis membrane (type VS, 0.025 µM from Millipore). The intactness of the purified P1 DNA insert is assessed by electrophoresis on 1% agarose (Sea Kem GTG; FMC Bio-products) pulse-field gel and staining with ethidium bromide.

Viral vectors

In one specific embodiment, the vector is derived from an adenovirus. Preferred adenovirus vectors according to the invention are those described by Feldman and Steg (1996), or Ohno *et al.*, (1994), which disclosures are hereby incorporated by reference in their entireties. Another preferred recombinant adenovirus according to this specific embodiment of the present invention is the human adenovirus type 2 or 5 (Ad 2 or Ad 5) or an adenovirus of animal origin (French patent application No. FR-93.05954), which disclosure is hereby incorporated by reference in its entirety.

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery systems of choice for the transfer of exogenous polynucleotides *in vivo*, particularly to mammals, including humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. Particularly preferred retroviruses for the preparation or construction of retroviral *in vitro* or *in vitro* gene

delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus and Rous Sarcoma virus. Particularly preferred Murine Leukemia Viruses include the 4070A and the 1504A viruses, Abelson (ATCC No VR-999), Friend (ATCC No VR-245), Gross (ATCC No VR-590), Rauscher (ATCC No VR-998) and Moloney Murine Leukemia Virus (ATCC No VR-190; PCT Application No WO 94/24298). Particularly preferred Rous Sarcoma Viruses include Bryan high titer (ATCC Nos VR -334, VR-657, VR-726, VR-659 and VR-728). Other preferred retroviral vectors are those described in Roth *et al.* (1996), PCT Application No WO 93/25234, PCT Application No WO 94/ 06920, Roux *et al.*, (1989), Julan *et al.*, (1992), and Neda *et al.*, (1991), which disclosures are hereby incorporated by reference in their entireties.

Yet another viral vector system that is contemplated by the invention comprises the adeno-associated virus (AAV). The adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka *et al.*, 1992), which disclosure is hereby incorporated by reference in its entirety. It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (Flotte *et al.* 1992; Samulski *et al.*, 1989; McLaughlin *et al.*, 1989), which disclosures are hereby incorporated by reference in their entireties. One advantageous feature of AAV derives from its reduced efficacy for transducing primary cells relative to transformed cells.

BAC vectors

The bacterial artificial chromosome (BAC) cloning system (Shizuya *et al.*, 1992), which disclosure is hereby incorporated by reference in its entirety, has been developed to stably maintain large fragments of genomic DNA (100-300 kb) in *E. coli*. A preferred BAC vector comprises a pBeloBAC11 vector that has been described by Kim *et al.* (1996), which disclosure is hereby incorporated by reference in its entirety. BAC libraries are prepared with this vector using size-selected genomic DNA that has been partially digested using enzymes that permit ligation into either the *Bam* HI or *Hind*III sites in the vector. Flanking these cloning sites are T7 and SP6 RNA polymerase transcription initiation sites that can be used to generate end probes by either RNA transcription or PCR methods. After the construction of a BAC library in *E. coli*, BAC DNA is purified from the host cell as a supercoiled circle. Converting these circular molecules into a linear form precedes both size determination and introduction of the BACs into recipient cells. The cloning site is flanked by two *Not* I sites, permitting cloned segments to be excised from the vector by *Not* I digestion. Alternatively, the DNA insert contained in the pBeloBAC11 vector may be linearized by treatment of the BAC vector with the commercially available enzyme lambda terminase that leads to the cleavage at the unique *cos*N site, but this cleavage method results in a full length BAC clone containing both the insert DNA and the BAC sequences.

Baculovirus:

Another specific suitable host vector system is the pVL1392/1393 baculovirus transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC No. CRL 1711) which is derived from *Spodoptera frugiperda*. Other suitable vectors for the expression of the FRADJ and/or CRYPTIC polypeptide of the present invention in a baculovirus expression system include those described by Chai *et al.*, (1993), Vlasak *et al.*, (1983), and Lenhard *et al.*, (1996), which disclosures are hereby incorporated by reference in their entireties.

Delivery Of The Recombinant Vectors:

To effect expression of the polynucleotides and polynucleotide constructs of the invention, these constructs must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cell lines, or *in vivo* or *ex vivo*, as in the treatment of certain diseases states. One mechanism is viral infection where the expression construct is encapsulated in an infectious viral particle.

Several non-viral methods for the transfer of polynucleotides into cultured mammalian cells are also contemplated by the present invention, and include, without being limited to, calcium phosphate precipitation (Graham *et al.*, 1973; Chen *et al.*, 1987); DEAE-dextran (Gopal, 1985); electroporation (Tur-Kaspa *et al.*, 1986; Potter *et al.*, 1984); direct microinjection (Harland *et al.*, 1985); DNA-loaded liposomes (Nicolau *et al.*, 1982; Fraley *et al.*, 1979); and gene-mediated transfection. (Wu and Wu, 1987, 1988), which disclosures are hereby incorporated by reference in their entireties. Some of these techniques may be successfully adapted for *in vivo* or *ex vivo* use.

Once the expression polynucleotide has been delivered into the cell, it may be stably integrated into the genome of the recipient cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle.

One specific embodiment for a method for delivering a protein or peptide to the interior of a cell of a vertebrate *in vivo* comprises the step of introducing a preparation comprising a physiologically acceptable carrier and a naked polynucleotide operatively coding for the polypeptide of interest into the interstitial space of a tissue comprising the cell, whereby the naked polynucleotide is taken up into the interior of the cell and has a physiological effect. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* as well.

Compositions for use *in vitro* and *in vivo* comprising a "naked" polynucleotide are described in PCT application No. WO 90/11092 (Vical Inc.) and also in PCT application No. WO 95/11307 (Institut

Pasteur, INSERM, Université d'Ottawa) as well as in the articles of Tacson *et al.* (1996) and of Huygen *et al.*, (1996), which disclosures are hereby incorporated by reference in their entireties.

In still another embodiment of the invention, the transfer of a naked polynucleotide of the invention, including a polynucleotide construct of the invention, into cells may be proceeded with a particle bombardment (biolistic), said particles being DNA-coated microprojectiles accelerated to a high velocity allowing them to pierce cell membranes and enter cells without killing them, such as described by Klein *et al.*, (1987), which disclosure is hereby incorporated by reference in its entirety.

In a further embodiment, the polynucleotide of the invention may be entrapped in a liposome (Ghosh and Bacchawat, 1991; Wong *et al.*, 1980; Nicolau *et al.*, 1987), which disclosures are hereby incorporated by reference in their entireties.

In a specific embodiment, the invention provides a composition for the *in vivo* production of the FRADJ and/or CRYPTIC protein or polypeptide described herein. It comprises a naked polynucleotide operatively coding for this polypeptide, in solution in a physiologically acceptable carrier, and suitable for introduction into a tissue to cause cells of the tissue to express the said protein or polypeptide.

The amount of vector to be injected to the desired host organism varies according to the site of injection. As an indicative dose, it will be injected between 0,1 and 100 µg of the vector in an animal body, preferably a mammal body, for example a mouse body.

In another embodiment of the vector according to the invention, it may be introduced *in vitro* in a host cell, preferably in a host cell previously harvested from the animal to be treated and more preferably a somatic cell such as a muscle cell. In a subsequent step, the cell that has been transformed with the vector coding for the desired FRADJ and/or CRYPTIC polypeptide or the desired fragment thereof is reintroduced into the animal body in order to deliver the recombinant protein within the body either locally or systemically.

Cell Hosts

Another object of the invention comprises a host cell that has been transformed or transfected with one of the polynucleotides described herein, and in particular a polynucleotide either comprising a FRADJ and/or CRYPTIC regulatory polynucleotide or the polynucleotide coding for a FRADJ and/or CRYPTIC polypeptide. Also included are host cells that are transformed (prokaryotic cells) or that are transfected (eukaryotic cells) with a recombinant vector such as one of those described above. However, the cell hosts of the present invention can comprise any of the polynucleotides of the present invention. In a preferred embodiment, host cells contain a polynucleotide sequence comprising a sequence selected from the group consisting of sequences of SEQ ID NO:1 or 3, sequences of clone inserts of the deposited clone pool, variants and fragments thereof. Preferred host cells used as recipients for the expression vectors of the invention are the following:

a) Prokaryotic host cells: *Escherichia coli* strains (I.E.DH5-α strain), *Bacillus subtilis*, *Salmonella typhimurium*, and strains from species like *Pseudomonas*, *Streptomyces* and *Staphylococcus*.

b) Eukaryotic host cells: HeLa cells (ATCC No.CCL2; No.CCL2.1; No.CCL2.2), Cv 1 cells (ATCC No.CCL70), COS cells (ATCC No.CRL1650; No.CRL1651), S f-9 cells (ATCC No.CRL1711), C127 cells (ATCC No. CRL-1804), 3T3 (ATCC No. CRL-6361), CHO (ATCC No. CCL-61), human kidney 293. (ATCC No. 45504; No. CRL-1573) and BHK (ECACC No. 84100501; No. 84111301).

c) Other mammalian host cells.

The present invention also encompasses primary, secondary, and immortalized homologously recombinant host cells of vertebrate origin, preferably mammalian origin and particularly human origin, that have been engineered to: a) insert exogenous (heterologous) polynucleotides into the endogenous chromosomal DNA of a targeted gene, b) delete endogenous chromosomal DNA, and/or c) replace endogenous chromosomal DNA with exogenous polynucleotides. Insertions, deletions, and/or replacements of polynucleotide sequences may be to the coding sequences of the targeted gene and/or to regulatory regions, such as promoter and enhancer sequences, operably associated with the targeted gene.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller *et al.*, (1989); and Zijlstra *et al.* (1989) (The disclosures of each of which are incorporated by reference in their entireties).

The present invention further relates to a method of making a homologously recombinant host cell *in vitro* or *in vivo*, wherein the expression of a targeted gene not normally expressed in the cell is altered. Preferably the alteration causes expression of the targeted gene under normal growth conditions or under conditions suitable for producing the polypeptide encoded by the targeted gene. The method comprises the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, said polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and

(b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination.

The present invention further relates to a method of altering the expression of a targeted gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, said polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and (c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene.

The present invention further relates to a method of making a polypeptide of the present invention by altering the expression of a targeted endogenous gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: a) transfecting the cell *in vitro* with a polynucleotide construct, said polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene thereby making the polypeptide.

The present invention further relates to a polynucleotide construct that alters the expression of a targeted gene in a cell type in which the gene is not normally expressed. This occurs when the polynucleotide construct is inserted into the chromosomal DNA of the target cell, wherein said polynucleotide construct comprises: a) a targeting sequence; b) a regulatory sequence and/or coding sequence; and c) an unpaired splice-donor site, if necessary. Further included are a polynucleotide construct, as described above, wherein said polynucleotide construct further comprises a polynucleotide which encodes a polypeptide and is in-frame with the targeted endogenous gene after homologous recombination with chromosomal DNA.

The compositions may be produced, and methods performed, by techniques known in the art, such as those described in U.S. Patent Nos: 6,054,288; 6,048,729; 6,048,724; 6,048,524; 5,994,127; 5,968,502; 5,965,125; 5,869,239; 5,817,789; 5,783,385; 5,733,761; 5,641,670; 5,580,734; International Publication Nos: WO 96/29411, WO 94/12650; and scientific articles described by Koller *et al.*, (1994). (The disclosures of each of which are incorporated by reference in their entireties).

The FRADJ and/or CRYPTIC gene expression in mammalian cells, preferably human cells, may be rendered defective, or alternatively may be altered by replacing the endogenous FRADJ and/or CRYPTIC gene in the genome of an animal cell by a FRADJ and/or CRYPTIC polynucleotide according

to the invention. These genetic alterations may be generated by homologous recombination using previously described specific polynucleotide constructs.

Mammal zygotes, such as murine zygotes may be used as cell hosts. For example, murine zygotes may undergo microinjection with a purified DNA molecule of interest, for example a purified DNA molecule that has previously been adjusted to a concentration ranging from 1 ng/ml –for BAC inserts- to 3 ng/µl –for P1 bacteriophage inserts- in 10 mM Tris-HCl, pH 7.4, 250 µM EDTA containing 100 mM NaCl, 30 µM spermine, and70 µM spermidine. When the DNA to be microinjected has a large size, polyamines and high salt concentrations can be used in order to avoid mechanical breakage of this DNA, as described by Schedl *et al* (1993b), which disclosure is hereby incorporated by reference in its entirety.

Any one of the polynucleotides of the invention, including the Polynucleotide constructs described herein, may be introduced in an embryonic stem (ES) cell line, preferably a mouse ES cell line. ES cell lines are derived from pluripotent, uncommitted cells of the inner cell mass of pre-implantation blastocysts. Preferred ES cell lines are the following: ES-E14TG2a (ATCC No.CRL-1821), ES-D3 (ATCC No.CRL1934 and No. CRL-11632), YS001 (ATCC No. CRL-11776), 36.5 (ATCC No. CRL-11116). ES cells are maintained in an uncommitted state by culture in the presence of growth-inhibited feeder cells that provide the appropriate signals to preserve this embryonic phenotype and serve as a matrix for ES cell adherence. Preferred feeder cells are primary embryonic fibroblasts that are established from tissue of day 13- day 14 embryos of virtually any mouse strain, that are maintained in culture, such as described by Abbondanzo *et al.* (1993) and are growth-inhibited by irradiation, such as described by Robertson (1987), or by the presence of an inhibitory concentration of LIF, such as described by Pease and Williams (1990), which disclosures are hereby incorporated by reference in their entireties.

The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by the skilled artisan.

Uses of polypeptides of the invention

Polypeptides of the present invention have uses that include therapeutics. In particular, the invention relates to the use of soluble fragments of FRADJ and/or CRYPTIC polypeptide in a method of

increasing body weight by blocking the activity of LIGAND. Further use of FRADJ and/or CRYPTIC polypeptide relate to its use as an immunogen for the generation of FRADJ and/or CRYPTIC antibody.

Uses of antibodies

Antibodies of the present invention have uses that include, but are not limited to, methods known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. An example of such use using immunoaffinity chromatography is given below. The antibodies of the present invention may be used either alone or in combination with other compositions. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of antigen-bearing substances, including the polypeptides of the present invention, in biological samples [*See, e.g.*, Harlow *et al.* (1988), incorporated by reference in the entirety]. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

The invention further relates to antibodies that act as AGONISTS or ANTAGONISTS of the polypeptides of the present invention. For example, the present invention includes antibodies that disrupt the FRADJ and/or CRYPTIC/LIGAND interactions with the polypeptides of the invention either partially or fully. Included are both FRADJ and/or CRYPTIC-specific antibodies and LIGAND-specific antibodies. Included are FRADJ and/or CRYPTIC-specific antibodies, which do not prevent LIGAND binding but prevent FRADJ and/or CRYPTIC activation. FRADJ and/or CRYPTIC activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also included are FRADJ and/or CRYPTIC-specific antibodies which both prevent LIGAND binding and FRADJ and/or CRYPTIC activation. Likewise, included are neutralizing antibodies that bind the LIGAND and prevent binding of the LIGAND to FRADJ and/or CRYPTIC, as well as antibodies that bind the LIGAND, thereby preventing FRADJ and/or CRYPTIC activation, but do not prevent the LIGAND from binding FRADJ and/or CRYPTIC. Further included are antibodies that activate FRADJ and/or CRYPTIC. These antibodies may act as AGONISTS for either all or less than all of the biological activities affected by LIGAND-mediated FRADJ and/or CRYPTIC activation. The antibodies may be specified as AGONISTS or ANTAGONISTS for biological activities comprising specific activities disclosed herein. The above antibody AGONISTS can be made using methods known in the art. *See e.g.*, WO 96/40281; US Patent 5,811,097; Deng *et al.* (1998); Chen *et al.* (1998); Harrop *et al.* (1998); Zhu *et al.* (1998); Yoon *et al.* (1998); Prat *et al.* (1998); Pitard *et al.* (1997); Liautard *et al.* (1997); Carlson *et al.* (1997); Taryman *et al.* (1995); Muller *et al.* (1998); Bartunek *et al.* (1996) (said references incorporated by reference in their entireties).

As discussed above, antibodies of the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art (*See, e.g.* Greenspan and Bona (1989) and Nissinoff (1991), which

disclosures are hereby incorporated by reference in their entireties). For example, antibodies which bind to and competitively inhibit polypeptide multimerization or binding of a polypeptide of the invention to LIGAND can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization or binding domain and, as a consequence, bind to and neutralize polypeptide or its LIGAND. Such neutralization anti-idiotypic antibodies can be used to bind a polypeptide of the invention or to bind its LIGANDS/receptors, and thereby block its biological activity.

Immunoaffinity Chromatography

Antibodies prepared as described herein are coupled to a support. Preferably, the antibodies are monoclonal antibodies, but polyclonal antibodies may also be used. The support may be any of those typically employed in immunoaffinity chromatography, including Sepharose CL-4B (Pharmacia, Piscataway, NJ), Sepharose CL-2B (Pharmacia, Piscataway, NJ), Affi-gel 10 (Biorad, Richmond, CA), or glass beads.

The antibodies may be coupled to the support using any of the coupling reagents typically used in immunoaffinity chromatography, including cyanogen bromide. After coupling the antibody to the support, the support is contacted with a sample that contains a target polypeptide whose isolation, purification or enrichment is desired. The target polypeptide may be a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 or 4 and polypeptides encoded by the clone inserts of the deposited clone pool, variants and fragments thereof, or a fusion protein comprising said selected polypeptide or a fragment thereof.

Preferably, the sample is placed in contact with the support for a sufficient amount of time and under appropriate conditions to allow at least 50% of the target polypeptide to specifically bind to the antibody coupled to the support.

Thereafter, the support is washed with an appropriate wash solution to remove polypeptides that have non-specifically adhered to the support. The wash solution may be any of those typically employed in immunoaffinity chromatography, including PBS, Tris-lithium chloride buffer (0.1M lysine base and 0.5M lithium chloride, pH 8.0), Tris-hydrochloride buffer (0.05M Tris-hydrochloride, pH 8.0), or Tris/Triton/NaCl buffer (50mM Tris.cl, pH 8.0 or 9.0, 0.1% Triton X-100, and 0.5MNaCl).

After washing, the specifically bound target polypeptide is eluted from the support using the high pH or low pH elution solutions typically employed in immunoaffinity chromatography. In particular, the elution solutions may contain an eluant such as triethanolamine, diethylamine, calcium chloride, sodium thiocyanate, potasssium bromide, acetic acid, or glycine. In some embodiments, the elution solution may also contain a detergent such as Triton X-100 or octyl-beta-D-glucoside.

Expression of FRADJ and/or CRYPTIC products

Spatial expression of the FRADJ and/or CRYPTIC genes of the invention

Tissue expression of the cDNA of the present invention was examined. The tissues and cell types examined for polynucleotide expression were: brain, fetal brain, fetal kidney, fetal liver, pituitary gland, liver, placenta, prostate, salivary gland, stomach/intestine, and testis. For each cDNA referred to by its sequence identification number (first column), the number of proprietary 5'ESTs (i.e. cDNA fragments) expressed in a particular tissue referred to by its name is indicated in parentheses (second column). In addition, the bias in the spatial distribution of the polynucleotide sequences of the present invention was examined by comparing the relative proportions of the biological polynucleotides of a given tissue using the following statistical analysis. The under- or over-representation of a polynucleotide of a given cluster in a given tissue was performed using the normal approximation of the binomial distribution. When the observed proportion of a polynucleotide of a given tissue in a given consensus had less than 1% chance to occur randomly according to the chi2 test, the frequency bias was reported as "preferred".

In addition, the spatial distribution of the polynucleotide sequences of the present invention was investigated using information from public databases. The expression of the polynucleotide of SEQ ID NO:1 and 3 was examined by comparing them to the polynucleotide sequences in public databases.

The cellular localization of some polypeptides of the invention was also determined using the "psort software" (Nakai, and Horton, (1999); Nakai and Kanehisa, (1992), which disclosures are hereby incorporated by reference in their entireties).

Evaluation of Expression Levels and Patterns of FRADJ and/or CRYPTIC mRNAs

The spatial and temporal expression patterns of FRADJ and/or CRYPTIC mRNAs, as well as their expression levels, may also be further determined as follows.

Expression levels and patterns of FRADJ and/or CRYPTIC mRNAs may be analyzed by solution hybridization with long probes as described in International Patent Application No. WO 97/05277, the entire contents of which are hereby incorporated by reference. Briefly, a FRADJ and/or CRYPTIC polynucleotide, or fragment thereof corresponding to the gene encoding the mRNA to be characterized is inserted at a cloning site immediately downstream of a bacteriophage (T3, T7 or SP6) RNA polymerase promoter to produce antisense RNA. Preferably, the FRADJ and/or CRYPTIC polynucleotide is at least a 100 nucleotides in length. The plasmid is linearized and transcribed in the presence of ribonucleotides comprising modified ribonucleotides (i.e. biotin-UTP and DIG-UTP). An excess of this doubly labeled RNA is hybridized in solution with mRNA isolated from cells or tissues of interest. The hybridizations are performed under standard stringent conditions (40-50°C for 16 hours in an 80% formamide, 0.4 M NaCl buffer, pH 7-8). The unhybridized probe is removed by digestion with ribonucleases specific for single-stranded RNA (i.e. RNases CL3, T1, Phy M, U2 or A). The presence of the biotin-UTP modification enables capture of the hybrid on a microtitration plate coated with streptavidin. The

presence of the DIG modification enables the hybrid to be detected and quantified by ELISA using an anti-DIG antibody coupled to alkaline phosphatase.

The FRADJ and/or CRYPTIC cDNA, or fragments thereof may also be tagged with nucleotide sequences for the serial analysis of gene expression (SAGE) as disclosed in UK Patent Application No. 2 305 241 A, the entire contents of which are incorporated by reference. In this method, cDNA are prepared from a cell, tissue, organism or other source of nucleic acid for which it is desired to determine gene expression patterns. The resulting cDNA are separated into two pools. The cDNA in each pool are cleaved with a first restriction endonuclease, called an "anchoring enzyme," having a recognition site that is likely to be present at least once in most cDNA. The fragments that contain the 5' or 3' most region of the cleaved cDNA are isolated by binding to a capture medium such as streptavidin coated beads. A first oligonucleotide linker having a first sequence for hybridization of an amplification primer and an internal restriction site for a "tagging endonuclease" is ligated to the digested cDNA in the first pool. Digestion with the second endonuclease produces short "tag" fragments from the cDNA. A second oligonucleotide having a second sequence for hybridization of an amplification primer and an internal restriction site is ligated to the digested cDNA in the second pool. The cDNA fragments in the second pool are also digested with the "tagging endonuclease" to generate short "tag" fragments derived from the cDNA in the second pool. The "tags" resulting from digestion of the first and second pools with the anchoring enzyme and the tagging endonuclease are ligated to one another to produce "ditags." In some embodiments, the ditags are concatamerized to produce ligation products containing from 2 to 200 ditags. The tag sequences are then determined and compared to the sequences of the FRADJ and/or CRYPTIC cDNA to determine which genes are expressed in the cell, tissue, organism, or other source of nucleic acids from which the tags were derived. In this way, the expression pattern of a FRADJ and/or CRYPTIC gene in the cell, tissue, organism, or other source of nucleic acids is obtained.

Quantitative analysis of FRADJ and/or CRYPTIC gene expression may also be performed using arrays. For example, quantitative analysis of gene expression may be performed with FRADJ and/or CRYPTIC polynucleotides, or fragments thereof in a complementary DNA microarray as described by Schena *et al.* (1995 and 1996) which disclosures are hereby incorporated by reference in their entireties. FRADJ and/or CRYPTIC cDNA or fragments thereof are amplified by PCR and arrayed from 96-well microtiter plates onto silylated microscope slides using high-speed robotics. Printed arrays are incubated in a humid chamber to allow rehydration of the array elements and rinsed, once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in sodium borohydride solution. The arrays are submerged in water for 2 min at 95°C, transferred into 0.2% SDS for 1 min, rinsed twice with water, air dried and stored in the dark at 25°C. Cell or tissue mRNA is isolated or commercially obtained and probes are prepared by a single round of reverse transcription. Probes are hybridized to 1 cm^2 microarrays under a 14 x 14 mm glass coverslip for 6-12 hours at 60°C. Arrays are washed for 5 min at 25°C in low stringency wash buffer (1X SSC/0.2% SDS), then for 10 min at room temperature in high stringency

wash buffer (0.1X SSC/0.2% SDS). Arrays are scanned in 0.1X SSC using a fluorescence laser scanning device fitted with a custom filter set. Accurate differential expression measurements are obtained by taking the average of the ratios of two independent hybridizations.

Quantitative analysis of the expression of genes may also be performed with FRADJ and/or CRYPTIC cDNA or fragments thereof in complementary DNA arrays as described by Pietu *et al.* (1996), which disclosure is hereby incorporated by reference in its entirety. The FRADJ and/or CRYPTIC polynucleotides of the invention or fragments thereof are PCR amplified and spotted on membranes. Then, mRNAs originating from various tissues or cells are labeled with radioactive nucleotides. After hybridization and washing in controlled conditions, the hybridized mRNAs are detected by phospho-imaging or autoradiography. Duplicate experiments are performed and a quantitative analysis of differentially expressed mRNAs is then performed.

Alternatively, expression analysis of FRADJ and/or CRYPTIC genes can be done through high density nucleotide arrays as described by Lockhart *et al.* (1996) and Sosnowski *et al.* (1997), which disclosures are hereby incorporated by reference in their entireties. Oligonucleotides of 15-50 nucleotides corresponding to sequences of a FRADJ and/or CRYPTIC polynucleotide or fragments thereof are synthesized directly on the chip (Lockhart *et al.*, supra) or synthesized and then addressed to the chip (Sosnowski *et al.*, supra). Preferably, the oligonucleotides are about 20 nucleotides in length. cDNA probes labeled with an appropriate compound, such as biotin, digoxigenin or fluorescent dye, are synthesized from the appropriate mRNA population and then randomly fragmented to an average size of 50 to 100 nucleotides. The said probes are then hybridized to the chip. After washing as described in Lockhart *et al.*, (supra) and application of different electric fields (Sosnowsky *et al.*, supra), the dyes or labeling compounds are detected and quantified. Duplicate hybridizations are performed. Comparative analysis of the intensity of the signal originating from cDNA probes on the same target oligonucleotide in different cDNA samples indicates a differential expression of the FRADJ and/or CRYPTIC mRNA.

Uses of FRADJ and/or CRYPTIC expression data

Once the expression levels and patterns of a FRADJ and/or CRYPTIC mRNA has been determined using any technique known to those skilled in the art. This information may be used to design FRADJ and/or CRYPTIC specific markers for detection, identification, screening and diagnosis purposes as well as to design DNA constructs with an expression pattern similar to a FRADJ and/or CRYPTIC expression pattern.

Detection of FRADJ and/or CRYPTIC expression and/or biological activity

The invention further relates to methods of detection of FRADJ and/or CRYPTIC expression and/or biological activity in a biological sample using the polynucleotide and polypeptide sequences

described herein. Such method scan be used, for example, as a screen for normal or abnormal FRADJ and/or CRYPTIC expression and/or biological activity and, thus, can be used diagnostically. The biological sample for use in the methods of the present invention includes a suitable sample from, for example, a mammal, particularly a human. For example, the sample can be issued from tissues or cell lines having the same origin as tissues or cell lines in which the polypeptide is known to be expressed.

Detection of FRADJ and/or CRYPTIC products

The invention further relates to methods of detection of FRADJ and/or CRYPTIC polynucleotides or polypeptides in a sample using the sequences described herein and any techniques known to those skilled in the art. For example, a labeled polynucleotide probe having all or a functional portion of the nucleotide sequence of a FRADJ and/or CRYPTIC polynucleotide can be used in a method to detect a FRADJ and/or CRYPTIC polynucleotide in a sample. In one embodiment, the sample is treated to render the polynucleotides in the sample available for hybridization to a polynucleotide probe, which can be DNA or RNA. The resulting treated sample is combined with a labeled polynucleotide probe having all or a portion of the nucleotide sequence of the FRADJ and/or CRYPTIC cDNA or genomic sequence, under conditions appropriate for hybridization of complementary sequences to occur. Detection of hybridization of polynucleotides from the sample with the labeled nucleic probe indicates the presence of FRADJ and/or CRYPTIC polynucleotides in a sample. The presence of FRADJ and/or CRYPTIC mRNA is indicative of FRADJ and/or CRYPTIC expression.

Consequently, the invention comprises methods for detecting the presence of a polynucleotide comprising a nucleotide sequence selected from a group consisting of the sequences of SEQ ID NO:1 and/or 3, the sequences of clone inserts of the deposited clone pool, sequences fully complementary thereto, fragments and variants thereof in a sample. In a first embodiment, said method comprises the following steps of:

a) bringing into contact said sample and a nucleic acid probe or a plurality of nucleic acid probes which hybridize to said selected nucleotide sequence; and

b) detecting the hybrid complex formed between said probe or said plurality of probes and said polynucleotide.

In a preferred embodiment of the above detection method, said nucleic acid probe or said plurality of nucleic acid probes is labeled with a detectable molecule. In another preferred embodiment of the above detection method, said nucleic acid probe or said plurality of nucleic acid probes has been immobilized on a substrate. In still another preferred embodiment, said nucleic acid probe or said plurality of nucleic acid probes has a sequence comprised in a sequence complementary to said selected sequence.

In a second embodiment, said method comprises the following steps of:

a) contacting said sample with amplification reaction reagents comprising a pair of amplification primers located on either side of the region of said nucleotide sequence to be amplified;

b) performing an amplification reaction to synthesize amplification products containing said region of said selected nucleotide sequence; and

c) detecting said amplification products.

In a preferred embodiment of the above detection method, when the polynucleotide to be amplified is a RNA molecule, preliminary reverse transcription and synthesis of a second cDNA strand are necessary to provide a DNA template to be amplified. In another preferred embodiment of the above detection method, the amplification product is detected by hybridization with a labeled probe having a sequence that is complementary to the amplified region. In still another preferred embodiment, at least one of said amplification primer has a sequence comprised in said selected sequence or in the sequence complementary to said selected sequence.

Alternatively, a method of detecting FRADJ and/or CRYPTIC expression in a test sample can be accomplished using any product that binds to a FRADJ and/or CRYPTIC polypeptide of the present invention or a portion of a FRADJ and/or CRYPTIC polypeptide. Such products may be antibodies, binding fragments of antibodies, polypeptides able to bind specifically to FRADJ and/or CRYPTIC polypeptides or fragments thereof, including FRADJ and/or CRYPTIC AGONISTS and ANTAGONISTS. Detection of specific binding to the antibody indicates the presence of a FRADJ and/or CRYPTIC polypeptide in the sample (e.g., ELISA).

Consequently, the invention is also directed to a method for detecting specifically the presence of a FRADJ and/or CRYPTIC polypeptide according to the invention in a biological sample, said method comprising the following steps of:

a) bringing into contact said biological sample with a product able to bind to a polypeptide of the invention or fragments thereof;

b) allowing said product to bind to said polypeptide to form a complex; and

c) detecting said complex.

In a preferred embodiment of the above detection method, the product is an antibody. In a more preferred embodiment, said antibody is labeled with a detectable molecule. In another more preferred embodiment of the above detection method, said antibody has been immobilized on a substrate.

In addition, the invention also relates to methods of determining whether a FRADJ and/or CRYPTIC product (e.g. a polynucleotide or polypeptide) is present or absent in a biological sample, said methods comprising the steps of:

a) obtaining said biological sample from a human or non-human animal, preferably a mammal;

b) contacting said biological sample with a product able to bind to a FRADJ and/or CRYPTIC polynucleotide or polypeptide of the invention; and

c) determining the presence or absence of said FRADJ and/or CRYPTIC product in said biological sample.

The present invention also relates to kits that can be used in the detection of FRADJ and/or CRYPTIC expression products. The kit can comprise a compound that specifically binds a FRADJ and/or CRYPTIC polypeptide (e.g. binding proteins, antibodies or binding fragments thereof (e.g. F(ab')2 fragments) or a FRADJ and/or CRYPTIC mRNA (e.g. a complementary probe or primer), for example, disposed within a container means. The kit can further comprise ancillary reagents, including buffers and the like.

The invention further includes methods of detecting specifically a FRADJ and/or CRYPTIC biological activity. Assessing the FRADJ and/or CRYPTIC biological activity may be performed by the detection of a change in cellular activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity.

Identification of a specific context of FRADJ and/or CRYPTIC expression

When the expression pattern of a FRADJ and/or CRYPTIC mRNA shows that a FRADJ and/or CRYPTIC gene is specifically expressed in a given context, probes and primers specific for this gene as well as antibodies binding to the FRADJ and/or CRYPTIC polynucleotide may then be used as markers for a specific context. Examples of specific contexts are: specific expression in a given tissue/cell or tissue/cell type, expression at a given stage of development of a process such as embryo development or disease development, or specific expression in a given organelle. Such primers, probes, and antibodies are useful commercially to identify tissues/cells/organelles of unknown origin, for example, forensic samples, differentiated tumor tissue that has metastasized to foreign bodily sites, or to differentiate different tissue types in a tissue cross-section using any technique known to those skilled in the art including *in situ* PCR or immunochemistry for example.

For example, the cDNA and proteins of the sequence listing and fragments thereof, may be used to distinguish human tissues/cells from non-human tissues/cells and to distinguish between human tissues/cells/organelles that do and do not express the polynucleotides comprising the cDNA. By knowing the expression pattern of a given gene, either through routine experimentation or by using the instant disclosure, the polynucleotides and polypeptides of the present invention may be used in methods of determining the identity of an unknown tissue/cell sample/organelle. As part of determining the identity of an unknown tissue/cell sample/organelle, the polynucleotides and polypeptides of the present

invention may be used to determine what the unknown tissue/cell sample is and what the unknown sample is not. For example, if a cDNA is expressed in a particular tissue/cell type/organelle, and the unknown tissue/cell sample/organelle does not express the cDNA, it may be inferred that the unknown tissue/cells are either not human or not the same human tissue/cell type/organelle as that which expresses the cDNA. These methods of determining tissue/cell/organelle identity are based on methods that detect the presence or absence of the mRNA (or corresponding cDNA) in a tissue/cell sample using methods well know in the art (e.g., hybridization, PCR based methods, immunoassays, immunochemistry, ELISA). Examples of such techniques are described in more detail below. Therefore, the invention encompasses uses of the polynucleotides and polypeptides of the invention as tissue markers. In a preferred embodiment, polynucleotides preferentially expressed in given tissues and polypeptides encoded by such polynucleotides are used for this purpose. The invention also encompasses uses of polypeptides of the invention as organelle markers. In a preferred embodiment, polypeptides preferentially expressed in given subcellular compartment are used for this purpose.

Consequently, the present invention encompasses methods of identification of a tissue/cell type/subcellular compartment, wherein said method includes the steps of:

a) contacting a biological sample which identity is to be assayed with a product able to bind a FRADJ and/or CRYPTIC product; and

b) determining whether a FRADJ and/or CRYPTIC product is expressed in said biological sample.

Products that are able to bind specifically to a FRADJ and/or CRYPTIC product, namely a FRADJ and/or CRYPTIC polypeptide or a FRADJ and/or CRYPTIC mRNA, include FRADJ and/or CRYPTIC binding proteins, antibodies or binding fragments thereof (e.g. F(ab')2 fragments), as well as FRADJ and/or CRYPTIC complementary probes and primers.

Step b) may be performed using any detection method known to those skilled in the art including those disclosed herein, especially in the section entitled "Detection of FRADJ and/or CRYPTIC expression and/or biological activity".

Identification of Tissue Types or Cell Species by Means of Labeled Tissue Specific Antibodies

Identification of specific tissues is accomplished by the visualization of tissue specific antigens by means of antibody preparations that are conjugated, directly (e.g., green fluorescent protein) or indirectly to a detectable marker. Selected labeled antibody species bind to their specific antigen binding partner in tissue sections, cell suspensions, or in extracts of soluble proteins from a tissue sample to provide a pattern for qualitative or semi-qualitative interpretation.

Antisera for these procedures must have a potency exceeding that of the native preparation, and for that reason, antibodies are concentrated to a mg/ml level by isolation of the gamma globulin fraction,

for example, by ion-exchange chromatography or by ammonium sulfate fractionation. Also, to provide the most specific antisera, unwanted antibodies, for example to common proteins, must be removed from the gamma globulin fraction, for example by means of insoluble immunoabsorbents, before the antibodies are labeled with the marker. Either monoclonal or heterologous antisera is suitable for either procedure.

A. Immunohistochemical Techniques

Purified, high-titer antibodies, prepared as described above, are conjugated to a detectable marker, as described, for example, by Fudenberg, (1980) or Rose *et al.*, (1980), which disclosures are hereby incorporated by reference in their entireties.

A fluorescent marker, either fluorescein or rhodamine, is preferred, but antibodies can also be labeled with an enzyme that supports a color producing reaction with a substrate, such as horseradish peroxidase. Markers can be added to tissue-bound antibody in a second step, as described below. Alternatively, the specific anti-tissue antibodies can be labeled with ferritin or other electron dense particles, and localization of the ferritin coupled antigen-antibody complexes achieved by means of an electron microscope. In yet another approach, the antibodies are radiolabeled, with, for example ^{125}I, and detected by overlaying the antibody treated preparation with photographic emulsion. Preparations to carry out the procedures can comprise monoclonal or polyclonal antibodies to a single protein or peptide identified as specific to a tissue type, for example, brain tissue, or antibody preparations to several antigenically distinct tissue specific antigens can be used in panels, independently or in mixtures, as required. Tissue sections and cell suspensions are prepared for immunohistochemical examination according to common histological techniques. Multiple cryostat sections (about 4 um, unfixed) of the unknown tissue and known control, are mounted and each slide covered with different dilutions of the antibody preparation. Sections of known and unknown tissues should also be treated with preparations to provide a positive control, a negative control, for example, pre-immune sera, and a control for non-specific staining, for example, buffer. Treated sections are incubated in a humid chamber for 30 min at room temperature, rinsed, then washed in buffer for 30-45 min. Excess fluid is blotted away, and the marker developed. If the tissue specific antibody was not labeled in the first incubation, it can be labeled at this time in a second antibody-antibody reaction, for example, by adding fluorescein- or enzyme-conjugated antibody against the immunoglobulin class of the antiserum-producing species, for example, fluorescein labeled antibody to mouse IgG. Such labeled sera are commercially available. The antigen found in the tissues by the above procedure can be quantified by measuring the intensity of color or fluorescence on the tissue section, and calibrating that signal using appropriate standards.

B. Identification of Tissue Specific Soluble Proteins

The visualization of tissue specific proteins and identification of unknown tissues from that procedure is carried out using the labeled antibody reagents and detection strategy as described for

immunohistochemistry; however the sample is prepared according to an electrophoretic technique to distribute the proteins extracted from the tissue in an orderly array on the basis of molecular weight for detection. A tissue sample is homogenized using a Virtis apparatus; cell suspensions are disrupted by Dounce homogenization or osmotic lysis, using detergents in either case as required to disrupt cell membranes, as is the practice in the art. Insoluble cell components such as nuclei, microsomes, and membrane fragments are removed by ultracentrifugation, and the soluble protein-containing fraction concentrated if necessary and reserved for analysis. A sample of the soluble protein solution is resolved into individual protein species by conventional SDS polyacrylamide electrophoresis as described, for example, by Davis *et al.*, Section 19-2 (1986), using a range of amounts of polyacrylamide in a set of gels to resolve the entire molecular weight range of proteins to be detected in the sample. A size marker is run in parallel for purposes of estimating molecular weights of the constituent proteins. Sample size for analysis is a convenient volume of from 5 to55 ul, and containing from about 1 to 100 ug protein. An aliquot of each of the resolved proteins is transferred by blotting to a nitrocellulose filter paper, a process that maintains the pattern of resolution. Multiple copies are prepared. The procedure, known as Western Blot Analysis, is well described in Davis *et al.*, (1986) Section 19-3. One set of nitrocellulose blots is stained with Coomassie Blue dye to visualize the entire set of proteins for comparison with the antibody bound proteins. The remaining nitrocellulose filters are then incubated with a solution of one or more specific antisera to tissue specific proteins prepared as described herein. In this procedure, as in procedure A above, appropriate positive and negative sample and reagent controls are run.

In either procedure A or B, a detectable label can be attached to the primary tissue antigen-primary antibody complex according to various strategies and permutations thereof. In a straightforward approach, the primary specific antibody can be labeled; alternatively, the unlabeled complex can be bound by a labeled secondary anti-IgG antibody. In other approaches, either the primary or secondary antibody is conjugated to a biotin molecule, which can, in a subsequent step, bind an avidin conjugated marker. According to yet another strategy, enzyme labeled or radioactive protein A, which has the property of binding to any IgG, is bound in a final step to either the primary or secondary antibody. The visualization of tissue specific antigen binding at levels above those seen in control tissues to one or more tissue specific antibodies, prepared from the FRADJ and/or CRYPTIC sequences identified from cDNA sequences, can identify tissues of unknown origin, for example, forensic samples, or differentiated tumor tissue that has metastasized to foreign bodily sites.

Screening and diagnosis of abnormal FRADJ and/or CRYPTIC expression and/or biological activity

Moreover, antibodies and/or primers specific for FRADJ and/or CRYPTIC expression may also be used to identify abnormal FRADJ and/or CRYPTIC expression and/or biological activity, and subsequently to screen and/or diagnose disorders associated with abnormal FRADJ and/or CRYPTIC expression. For example, a particular disease may result from lack of expression, over expression, or

under expression of a FRADJ and/or CRYPTIC mRNA. By comparing mRNA expression patterns and quantities in samples taken from healthy individuals with those from individuals suffering from a particular disorder, genes responsible for this disorder may be identified. Primers, probes and antibodies specific for this FRADJ and/or CRYPTIC may then be used to elaborate kits of screening and diagnosis for a disorder in which the gene of interest is specifically expressed or in which its expression is specifically dysregulated, i.e. underexpressed or overexpressed.

Screening for specific disorders

The present invention also relates to methods of identifying individuals having elevated or reduced levels of FRADJ and/or CRYPTIC, which individuals are likely to benefit from therapies to suppress or enhance FRADJ and/or CRYPTIC expression, respectively. One example of such methods comprises the steps of:

a) obtaining from a mammal a biological sample;

b) detecting the presence in said sample of a FRADJ and/or CRYPTIC product (mRNA or protein);

c) comparing the amount of said FRADJ and/or CRYPTIC product present in said sample with that of a control sample; and

d) determining whether said human or non-human mammal has a reduced or elevated level of FRADJ and/or CRYPTIC expression compared to the control sample.

A biological sample from a subject affected by obesity-related disease/disorder or a disorder associated with excessive weight loss can be screened for the presence of abnormal levels of FRADJ and/or CRYPTIC gene product. Obesity-related diseases and disorders include, but are not limited to, obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders include hyperlipidemia and hyperuricemia. Disorders associated with excessive weight loss include, but are not limited to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia. A biological sample from said subjects can be screened for the presence of abnormal levels of FRADJ and/or CRYPTIC gene product, the presence of abnormal levels of the gene product, relative to a normal population (standard), being indicative of predisposition to or as a present indication of the aforesaid obesity-related diseases/disorders or disorders associated with excessive weight loss. Such individuals would be candidates for therapies. The identification of elevated

levels of FRADJ and/or CRYPTIC in a symptomatic patient would be indicative of an individual that would benefit from treatment with agents that suppress FRADJ and/or CRYPTIC expression or activity. The identification of low levels of FRADJ and/or CRYPTIC in a symptomatic patient would be indicative of an individual that would benefit from agents that induce FRADJ and/or CRYPTIC expression or activity.

Biological samples suitable for use in this method include biological fluids such as blood, syovial fluid, and cerebrospinal fluid. Tissue samples (e.g. biopsies) can also be used in the method of the invention, including samples derived from tissues including, but not restricted, to muscle, adipose tissue, blood, and liver. Cell cultures or cell extracts derived, for example, from tissue biopsies can also be used. The detection step of the present method can be performed using standard protocols for protein/mRNA detection. Examples of suitable protocols include Northern blot analysis, immunoassays (e.g. RIA, Western blots, immunohistochemical analyses), and PCR.

Thus, the present invention further relates to methods of identifying individuals or non-human animals at increased risk for developing, or present state of having, certain diseases/disorders associated with FRADJ and/or CRYPTIC abnormal expression or biological activity. Said diseases/disorders include, but are not restricted to, obesity-related diseases/disorders and disorders associated with excessive weight loss. Obesity and obesity-related diseases and disorders include, but are not restricted to, obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications include, but are not restricted to, microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders include, but are not restricted to, hyperlipidemia and hyperuricemia. Disorders associated with excessive weight loss include, but are not restricted to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia. One example of such methods comprises the steps of:

a) obtaining from a human or non-human mammal a biological sample;

b) detecting the presence in said sample of a FRADJ and/or CRYPTIC product (mRNA or protein);

c) comparing the amount of said FRADJ and/or CRYPTIC product present in said sample with that of a control sample; and

d) determining whether said human or non-human mammal is at increased risk for developing, or present state of having, a diseases or disorder.

In preferred embodiments, the biological sample is taken from animals presenting: symptoms analogous to human aforesaid obesity-related disease/disorder and disorders associated with excessive weight loss. In accordance with this method, the presence in the sample of altered levels of FRADJ and/or CRYPTIC product indicates that the subject is predisposed to the above-indicated diseases/disorders. Biological samples suitable for use in this method include biological fluids such as blood, synovial fluid, and cerebrospinal fluid. Tissue samples (e.g. biopsies) can also be used in the method of the invention, including samples derived from liver, muscle, adipose tissue, and blood. Cell cultures or cell extracts derived, for example, from tissue biopsies can also be used.

The diagnostic methodologies described herein are applicable to both humans and non-human mammals.

Detection of FRADJ and/or CRYPTIC mutations

The invention also encompasses methods to detect mutations in FRADJ and/or CRYPTIC polynucleotides of the invention. Such methods may advantageously be used to detect mutations occurring in FRADJ and/or CRYPTIC genes and preferably in their regulatory regions. When the mutation was proven to be associated with a disease or disorder such as obesity-related diseases/disorders or disorders associated with excessive weight loss, screening for such mutations may be used for screening and diagnosis purposes. Obesity and obesity-related diseases and disorders include, but are not restricted to, obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications include, but are not restricted to, microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders include, but are not restricted to, hyperlipidemia and hyperuricemia. Disorders associated with excessive weight loss include, but are not restricted to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

In one embodiment of the oligonucleotide arrays of the invention, an oligonucleotide probe matrix may advantageously be used to detect mutations occurring in FRADJ and/or CRYPTIC genes and preferably in their regulatory regions. For this particular purpose, probes are specifically designed to have a nucleotide sequence allowing their hybridization to the genes that carry known mutations (either by deletion, insertion or substitution of one or several nucleotides). By known mutations, it is meant, mutations on the FRADJ and/or CRYPTIC genes that have been identified according, for example to the technique used by Huang *et al.* (1996) or Samson *et al.* (1996), which disclosures are hereby incorporated by reference in their entireties.

Another technique that is used to detect mutations in FRADJ and/or CRYPTIC genes is the use of a high-density DNA array. Each oligonucleotide probe constituting a unit element of the high density DNA array is designed to match a specific subsequence of a FRADJ and/or CRYPTIC genomic DNA or cDNA. Thus, an array consisting of oligonucleotides complementary to subsequences of the target gene sequence is used to determine the identity of the target sequence with the wild gene sequence, measure its amount, and detect differences between the target sequence and the reference wild gene sequence of the FRADJ and/or CRYPTIC gene. In one such design, termed 4L tiled array, is implemented a set of four probes (A, C, G, T), preferably 15-nucleotide oligomers. In each set of four probes, the perfect complement will hybridize more strongly than mismatched probes. Consequently, a nucleic acid target of length L is scanned for mutations with a tiled array containing 4L probes, the whole probe set containing all the possible mutations in the known wild reference sequence. The hybridization signals of the 15-mer probe set tiled array are perturbed by a single base change in the target sequence. As a consequence, there is a characteristic loss of signal or a "footprint" for the probes flanking a mutation position. This technique was described by Chee *et al.* in 1996, which disclosure is hereby incorporated by reference in its entirety.

Construction of DNA constructs with a FRADJ and/or CRYPTIC expression pattern

In addition, characterization of the spatial and temporal expression patterns and expression levels of FRADJ and/or CRYPTIC mRNAs is also useful for constructing expression vectors capable of producing a desired level of gene product in a desired spatial or temporal manner, as discussed below.

DNA Construct That Enables Directing Temporal And Spatial FRADJ and/or CRYPTIC Gene Expression In Recombinant Cell Hosts And In Transgenic Animals

In order to study the physiological and phenotypic consequences of a lack of synthesis of a FRADJ and/or CRYPTIC protein, both at the cell level and at the multi cellular organism level, the invention also encompasses DNA constructs and recombinant vectors enabling a conditional expression of a specific allele of a FRADJ and/or CRYPTIC genomic sequence or cDNA and also of a copy of this genomic sequence or cDNA harboring substitutions, deletions, or additions of one or more bases as regards to a nucleotide sequence selected from the group consisting of sequences of SEQ ID No 1 or 3 and sequences of clone inserts of the deposited clone pool, or a fragment thereof, these base substitutions, deletions or additions being located either in an exon, an intron or a regulatory sequence, but preferably in the 5'-regulatory sequence or in an exon of the FRADJ and/or CRYPTIC genomic sequence or within the FRADJ and/or CRYPTIC cDNA.

A first preferred DNA construct is based on the tetracycline resistance operon *tet* from *E. coli* transposon Tn10 for controlling the FRADJ and/or CRYPTIC gene expression, such as described by Gossen *et al.* (1992, 1995) and Furth *et al.* (1994), which disclosures are hereby incorporated by

reference in their entireties. Such a DNA construct contains seven *tet* operator sequences from Tn10 (*tet*op) that are fused to either a minimal promoter or a 5'-regulatory sequence of the FRADJ and/or CRYPTIC gene, said minimal promoter or said FRADJ and/or CRYPTIC regulatory sequence being operably linked to a polynucleotide of interest that codes either for a sense or an antisense oligonucleotide or for a polypeptide, including a FRADJ and/or CRYPTIC polypeptide or a peptide fragment thereof. This DNA construct is functional as a conditional expression system for the nucleotide sequence of interest when the same cell also comprises a nucleotide sequence coding for either the wild type (tTA) or the mutant (rTA) repressor fused to the activating domain of viral protein VP16 of herpes simplex virus, placed under the control of a promoter, such as the HCMVIE1 enhancer/promoter or the MMTV-LTR. Indeed, a preferred DNA construct of the invention comprise both the polynucleotide containing the *tet* operator sequences and the polynucleotide containing a sequence coding for the tTA or the rTA repressor. In a specific embodiment, the conditional expression DNA construct contains the sequence encoding the mutant tetracycline repressor rTA, the expression of the polynucleotide of interest is silent in the absence of tetracycline and induced in its presence.

DNA Constructs Allowing Homologous Recombination: Replacement Vectors

A second preferred DNA construct will comprise, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the FRADJ and/or CRYPTIC genomic sequence; (b) a nucleotide sequence comprising a positive selection marker, such as the marker for neomycine resistance (*neo*); and (c) a second nucleotide sequence that is comprised in the FRADJ and/or CRYPTIC genomic sequence, and is located on the genome downstream the first FRADJ and/or CRYPTIC nucleotide sequence (a).

In a preferred embodiment, this DNA construct also comprises a negative selection marker located upstream the nucleotide sequence (a) or downstream the nucleotide sequence (c). Preferably, the negative selection marker comprises the thymidine kinase (*tk*) gene (Thomas *et al.*, 1986), the hygromycine beta gene (Te Riele *et al.*, 1990), the *hprt* gene (Van der Lugt *et al.*, 1991; Reid *et al.*, 1990) or the Diphteria toxin A fragment (*Dt-A*) gene (Nada *et al.*, 1993; Yagi *et al.*1990), which disclosures are hereby incorporated by reference in their entireties. Preferably, the positive selection marker is located within a FRADJ and/or CRYPTIC exon sequence so as to interrupt the sequence encoding a FRADJ and/or CRYPTIC protein. These replacement vectors are described, for example, by Thomas *et al.* (1986; 1987), Mansour *et al.* (1988) and Koller *et al.* (1992).

The first and second nucleotide sequences (a) and (c) may be indifferently located within a FRADJ and/or CRYPTIC regulatory sequence, an intronic sequence, an exon sequence or a sequence containing both regulatory and/or intronic and/or exon sequences. The size of the nucleotide sequences (a) and (c) ranges from 1 to 50 kb, preferably from 1 to 10 kb, more preferably from 2 to 6 kb and most preferably from 2 to 4 kb.

DNA Constructs Allowing Homologous Recombination: Cre-LoxP System

These new DNA constructs make use of the site specific recombination system of the P1 phage. The P1 phage possesses a recombinase called Cre that interacts specifically with a 34 base pairs *lox*P site. The *lox*P site is composed of two palindromic sequences of 13 bp separated by a 8 bp conserved sequence (Hoess *et al.*, 1986), which disclosure is hereby incorporated by reference in its entirety. The recombination by the Cre enzyme between two *lox*P sites having an identical orientation leads to the deletion of the DNA fragment.

The Cre-*lox*P system used in combination with a homologous recombination techni que has been first described by Gu *et al.* (1993, 1994), which disclosures are hereby incorporated by reference in their entireties. Briefly, a nucleotide sequence of interest to be inserted in a targeted location of the genome harbors at least two *lox*P sites in the same orientation and located at the respective ends of a nucleotide sequence to be excised from the recombinant genome. The excision event requires the presence of the recombinase (Cre) enzyme within the nucleus of the recombinant cell host. The recombinase enzyme may be brought at the desired time either by (a) incubating the recombinant cell hosts in a culture medium containing this enzyme, by injecting the Cre enzyme directly into the desired cell, such as described by Araki *et al.* (1995), which disclosure is hereby incorporated by reference in its entirety, or by lipofection of the enzyme into the cells, such as described by Baubonis *et al.* (1993), which disclosure is hereby incorporated by reference in its entirety; (b) transfecting the cell host with a vector comprising the *Cre* coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter being optionally inducible, said vector being introduced in the recombinant cell host, such as described by Gu *et al.* (1993) and Sauer *et al.* (1988), which disclosures are hereby incorporated by reference in their entireties; (c) introducing in the genome of the cell host a polynucleotide comprising the *Cre* coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter is optionally inducible, and said polynucleotide being inserted in the genome of the cell host either by a random insertion event or an homologous recombination event, such as described by Gu *et al.* (1994).

In a specific embodiment, the vector containing the sequence to be inserted in the FRADJ and/or CRYPTIC gene by homologous recombination is constructed in such a way that selectable markers are flanked by *lox*P sites of the same orientation, it is possible, by treatment by the Cre enzyme, to eliminate the selectable markers while leaving the FRADJ and/or CRYPTIC sequences of interest that have been inserted by an homologous recombination event. Again, two selectable markers are needed: a positive selection marker to select for the recombination event and a negative selection marker to select for the homologous recombination event. Vectors and methods using the Cre-*lox*P system are described by Zou *et al.* (1994), which disclosure is hereby incorporated by reference in its entirety.

Thus, a third preferred DNA construct of the invention comprises, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the FRADJ and/or CRYPTIC genomic sequence; (b) a nucleotide sequence comprising a polynucleotide encoding a positive selection marker, said nucleotide sequence comprising additionally two sequences defining a site recognized by a recombinase, such as a *lox*P site, the two sites being placed in the same orientation; and (c) a second nucleotide sequence that is comprised in the FRADJ and/or CRYPTIC genomic sequence, and is located on the genome downstream of the first FRADJ and/or CRYPTIC nucleotide sequence (a).

The sequences defining a site recognized by a recombinase, such as a *lox*P site, are preferably located within the nucleotide sequence (b) at suitable locations bordering the nucleotide sequence for which the conditional excision is sought. In one specific embodiment, two *lox*P sites are located at each side of the positive selection marker sequence, in order to allow its excision at a desired time after the occurrence of the homologous recombination event.

In a preferred embodiment of a method using the third DNA construct described above, the excision of the polynucleotide fragment bordered by the two sites recognized by a recombinase, preferably two loxP sites, is performed at a desired time, due to the presence within the genome of the recombinant host cell of a sequence encoding the Cre enzyme operably linked to a promoter sequence, preferably an inducible promoter, more preferably a tissue-specific promoter sequence and most preferably a promoter sequence which is both inducible and tissue-specific, such as described by Gu *et al.* (1994).

The presence of the Cre enzyme within the genome of the recombinant cell host may result from the breeding of two transgenic animals, the first transgenic animal bearing the FRADJ and/or CRYPTIC-derived sequence of interest containing the *lox*P sites as described above and the second transgenic animal bearing the *Cre* coding sequence operably linked to a suitable promoter sequence, such as described by Gu *et al.* (1994).

Spatio-temporal control of the Cre enzyme expression may also be achieved with an adenovirus based vector that contains the Cre gene thus allowing infection of cells, or *in vivo* infection of organs, for delivery of the Cre enzyme, such as described by Anton and Graham (1995) and Kanegae *et al.* (1995), which disclosures are hereby incorporated by reference in their entireties.

The DNA constructs described above may be used to introduce a desired nucleotide sequence of the invention, preferably a FRADJ and/or CRYPTIC genomic sequence or a FRADJ and/or CRYPTIC cDNA sequence, and most preferably an altered copy of a FRADJ and/or CRYPTIC genomic or cDNA sequence, within a predetermined location of the targeted genome, leading either to the generation of an altered copy of a targeted gene (knock-out homologous recombination) or to the replacement of a copy of the targeted gene by another copy sufficiently homologous to allow an homologous recombination event to occur (knock-in homologous recombination).

Modifying FRADJ and/or CRYPTIC expression and/or biological activity

Modifying endogenous FRADJ and/or CRYPTIC expression and/or biological activity is expressly contemplated by the present invention.

Screening for compounds that modulate FRADJ and/or CRYPTIC expression and/or biological activity

The present invention further relates to compounds able to modulate FRADJ and/or CRYPTIC expression and/or biological activity and methods to use these compounds. Such compounds may interact with the regulatory sequences of FRADJ and/or CRYPTIC genes or they may interact with FRADJ and/or CRYPTIC polypeptides directly or indirectly.

Compounds Interacting With FRADJ and/or CRYPTIC Regulatory Sequences

The present invention also concerns a method for screening substances or molecules that are able to interact with the regulatory sequences of a FRADJ and/or CRYPTIC gene, such as for example promoter or enhancer sequences in untranscribed regions of the genomic DNA, as determined using any techniques known to those skilled in the art including, or such as regulatory sequences located in untranslated regions of FRADJ and/or CRYPTIC mRNA.

Sequences within untranscribed or untranslated regions of polynucleotides of the invention may be identified by comparison to databases containing known regulatory sequence such as transcription start sites, transcription factor binding sites, promoter sequences, enhancer sequences, 5'UTR and 3'UTR elements (Pesole *et al.*, 2000; http://igs-server.cnrs-mrs.fr/~gauthere/UTR/index.html). Alternatively, the regulatory sequences of interest may be identified through conventional mutagenesis or deletion analyses of reporter plasmids.

Following the identification of potential FRADJ and/or CRYPTIC regulatory sequences, proteins that interact with these regulatory sequences may be identified as described below.

Gel retardation assays may be performed independently in order to screen candidate molecules that are able to interact with the regulatory sequences of the FRADJ and/or CRYPTIC gene, such as described by Fried and Crothers (1981), Garner and Revzin (1981) and Dent and Latchman (1993), the teachings of these publications being herein incorporated by reference. These techniques are based on the principle according to which a DNA or mRNA fragment that is bound to a protein migrates slower than the same unbound DNA or mRNA fragment. Briefly, the target nucleotide sequence is labeled. Then the labeled target nucleotide sequence is brought into contact with either a total nuclear extract from cells containing regulation factors, or with different candidate molecules to be tested. The interaction between the target regulatory sequence of the FRADJ and/or CRYPTIC gene and the

candidate molecule or the regulation factor is detected after gel or capillary electrophoresis through retardation in the migration.

Nucleic acids encoding proteins which are able to interact with the promoter sequence of the FRADJ and/or CRYPTIC gene, more particularly a nucleotide sequence selected from the group consisting of the polynucleotides of the 5' and 3' regulatory region or a fragment or variant thereof, may be identified by using a one-hybrid system, such as that described in the booklet enclosed in the Matchmaker One-Hybrid System kit from Clontech (Catalog Ref. n° K1603-1), the technical teachings of which are herein incorporated by reference. Briefly, the target nucleotide sequence is cloned upstream of a selectable reporter sequence and the resulting polynucleotide construct is integrated in the yeast genome (*Saccharomyces cerevisiae*). Preferably, multiple copies of the target sequences are inserted into the reporter plasmid in tandem. The yeast cells containing the reporter sequence in their genome are then transformed with a library comprising fusion molecules between cDNA encoding candidate proteins for binding onto the regulatory sequences of the FRADJ and/or CRYPTIC gene and sequences encoding the activator domain of a yeast transcription factor such as GAL4. The recombinant yeast cells are plated in a culture broth for selecting cells expressing the reporter sequence. The recombinant yeast cells thus selected contain a fusion protein that is able to bind onto the target regulatory sequence of the FRADJ and/or CRYPTIC gene. Then, the cDNA encoding the fusion proteins are sequenced and may be cloned into expression or transcription vectors *in vitro*. The binding of the encoded polypeptides to the target regulatory sequences of the FRADJ and/or CRYPTIC gene may be confirmed by techniques familiar to the one skilled in the art, such as gel retardation assays or DNAse protection assays.

Ligands interacting with FRADJ and/or CRYPTIC polypeptides

For the purpose of the present invention, a Ligand means a molecule, such as a protein, a peptide, an antibody or any synthetic chemical compound capable of binding to a FRADJ and/or CRYPTIC protein or one of its fragments or variants or to modulate the expression of the polynucleotide coding for FRADJ and/or CRYPTIC or a fragment or variant thereof.

In the Ligand screening method according to the present invention, a biological sample or a defined molecule to be tested as a putative Ligand of a FRADJ and/or CRYPTIC protein is brought into contact with the corresponding purified FRADJ and/or CRYPTIC protein, for example the corresponding purified recombinant FRADJ and/or CRYPTIC protein produced by a recombinant cell host as described herein, in order to form a complex between this protein and the putative Ligand molecule to be tested.

As an illustrative example, to study the interaction of a FRADJ and/or CRYPTIC protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 or 4 and polypeptides encoded by the clone inserts of the deposited clone pool, with drugs or small molecules, such as molecules generated through combinatorial

chemistry approaches, the microdialysis coupled to HPLC method described by Wang *et al.* (1997) or the affinity capillary electrophoresis method described by Bush *et al.* (1997), the disclosures of which are incorporated by reference, can be used.

In further methods, peptides, drugs, fatty acids, lipoproteins, or small molecules which interact with a FRADJ and/or CRYPTIC protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 or 4 and polypeptides encoded by the clone inserts of the deposited clone pool may be identified using assays such as the following. The molecule to be tested for binding is labeled with a detectable label, such as a fluorescent, radioactive, or enzymatic tag and placed in contact with immobilized FRADJ and/or CRYPTIC protein, or a fragment thereof under conditions that permit specific binding to occur. After removal of non-specifically bound molecules, bound molecules are detected using appropriate means.

Various candidate substances or molecules can be assayed for interaction with a FRADJ and/or CRYPTIC polypeptide. These substances or molecules include, without being limited to, natural or synthetic organic compounds or molecules of biological origin such as polypeptides. When the candidate substance or molecule comprises a polypeptide, this polypeptide may be the resulting expression product of a phage clone belonging to a phage-based random peptide library, or alternatively the polypeptide may be the resulting expression product of a cDNA library cloned in a vector suitable for performing a two-hybrid screening assay.

A. Candidate Ligands obtained from random peptide libraries

In a particular embodiment of the screening method, the putative Ligand is the expression product of a DNA insert contained in a phage vector (Parmley and Smith, 1988). Specifically, random peptide phages libraries are used. The random DNA inserts encode for peptides of 8 to 20 amino acids in length (Oldenburg *et al.*, 1992; Valadon *et al.*, 1996; Lucas, 1994; Westerink, 1995; Felici *et al.*, 1991), which disclosures are hereby incorporated by reference in their entireties. According to this particular embodiment, the recombinant phages expressing a protein that binds to an immobilized FRADJ and/or CRYPTIC protein is retained and the complex formed between the FRADJ and/or CRYPTIC protein and the recombinant phage may be subsequently immunoprecipitated by a polyclonal or a monoclonal antibody directed against the FRADJ and/or CRYPTIC protein.

Once the Ligand library in recombinant phages has been constructed, the phage population is brought into contact with the immobilized FRADJ and/or CRYPTIC protein. Then the preparation of complexes is washed in order to remove the non-specifically bound recombinant phages. The phages that bind specifically to the FRADJ and/or CRYPTIC protein are then eluted by a buffer (acid pH) or immunoprecipitated by the monoclonal antibody produced by the hybridoma anti-FRADJ and/or CRYPTIC, and this phage population is subsequently amplified by an over-infection of bacteria (for

example E. coli). The selection step may be repeated several times, preferably 2-4 times, in order to select the more specific recombinant phage clones. The last step comprises characterizing the peptide produced by the selected recombinant phage clones either by expression in infected bacteria and isolation, expressing the phage insert in another host-vector system, or sequencing the insert contained in the selected recombinant phages.

B. Candidate Ligands obtained by competition experiments.

Alternatively, peptides, drugs or small molecules which bind to a FRADJ and/or CRYPTIC protein or fragment thereof comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2, 4 and polypeptides encoded by the clone inserts of the deposited clone pool, may be identified in competition experiments. In such assays, the FRADJ and/or CRYPTIC protein, or a fragment thereof, is immobilized to a surface, such as a plastic plate. Increasing amounts of the peptides, drugs or small molecules are placed in contact with the immobilized FRADJ and/or CRYPTIC protein, or a fragment thereof, in the presence of a detectable labeled known FRADJ and/or CRYPTIC protein Ligand. For example, the FRADJ and/or CRYPTIC Ligand may be detectably labeled with a fluorescent, radioactive, or enzymatic tag. The ability of the test molecule to bind the FRADJ and/or CRYPTIC protein, or a fragment thereof, is determined by measuring the amount of detectably labeled known Ligand bound in the presence of the test molecule. A decrease in the amount of known Ligand bound to the FRADJ and/or CRYPTIC protein, or a fragment thereof, when the test molecule is present indicated that the test molecule is able to bind to the FRADJ and/or CRYPTIC protein, or a fragment thereof.

C. Candidate Ligands obtained by affinity chromatography.

Proteins or other molecules interacting with a FRADJ and/or CRYPTIC protein, or a fragment thereof comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 or 4 and polypeptides encoded by the clone inserts of the deposited clone pool, can also be found using affinity columns which contain the FRADJ and/or CRYPTIC protein, or a fragment thereof. The FRADJ and/or CRYPTIC protein, or a fragment thereof, may be attached to the column using conventional techniques including chemical coupling to a suitable column matrix such as agarose, Affi Gel®, or other matrices familiar to those of skill in art. In some embodiments of this method, the affinity column contains chimeric proteins in which the FRADJ and/or CRYPTIC protein, or a fragment thereof, is fused to glutathion S transferase (GST). A mixture of cellular proteins or pool of expressed proteins as described above is applied to the affinity column. Proteins or other molecules interacting with the FRADJ and/or CRYPTIC protein, or a fragment thereof, attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in

Ramunsen *et al.* (1997), the disclosure of which is incorporated by reference. Alternatively, the proteins retained on the affinity column can be purified by electrophoresis based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

D. Candidate Ligands obtained by optical biosensor methods

Proteins interacting with a FRADJ and/or CRYPTIC protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2, 4 and polypeptides encoded by the clone inserts of the deposited clone pool, can also be screened by using an Optical Biosensor as described in Edwards and Leatherbarrow (1997) and also in Szabo *et al.* (1995), the disclosures of which are incorporated by reference. This technique permits the detection of interactions between molecules in real time, without the need of labeled molecules. This technique is based on the surface plasmon resonance (SPR) phenomenon. Briefly, the candidate Ligand molecule to be tested is attached to a surface (such as a carboxymethyl dextran matrix). A light beam is directed towards the side of the surface that does not contain the sample to be tested and is reflected by said surface. The SPR phenomenon causes a decrease in the intensity of the reflected light with a specific association of angle and wavelength. The binding of candidate Ligand molecules cause a change in the refraction index on the surface, which change is detected as a change in the SPR signal. For screening of candidate Ligand molecules or substances that are able to interact with the FRADJ and/or CRYPTIC protein, or a fragment thereof, the FRADJ and/or CRYPTIC protein, or a fragment thereof, is immobilized onto a surface. This surface comprises one side of a cell through which flows t he candidate molecule to be assayed. The binding of the candidate molecule on the FRADJ and/or CRYPTIC protein, or a fragment thereof, is detected as a change of the SPR signal. The candidate molecules tested may be proteins, peptides, carbohydrates, lipids, or small molecules generated by combinatorial chemistry. This technique may also be performed by immobilizing eukaryotic or prokaryotic cells or lipid vesicles exhibiting an endogenous or a recombinantly expressed FRADJ and/or CRYPTIC protein at their surface.

The main advantage of the method is that it allows the determination of the association rate between the FRADJ and/or CRYPTIC protein and molecules interacting with the FRADJ and/or CRYPTIC protein. It is thus possible to select specifically Ligand molecules interacting with the FRADJ and/or CRYPTIC protein, or a fragment thereof, through strong or conversely weak association constants.

E. Candidate Ligands obtained through a two-hybrid screening assay.

The yeast two-hybrid system is designed to study protein-protein interactions *in vivo* (Fields and Song, 1989), which disclosure is hereby incorporated by reference in its entirety, and relies upon the

fusion of a bait protein to the DNA binding domain of the yeast Gal4 protein. This technique is also described in the US Patent N° US 5,667,973 and the US Patent N° 5,283,173, the technical teachings of both patents being herein incorporated by reference.

The general procedure of library screening by the two-hybrid assay may be performed as described by Harper *et al.* (1993) or as described by Cho *et al.* (1998) or also Fromont-Racine *et al.* (1997), which disclosures are hereby incorporated by reference in their entireties.

The bait protein or polypeptide comprises, consists essentially of, or consists of a FRADJ and/or CRYPTIC polypeptide or a fragment thereof comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 or 4 and polypeptides encoded by the clone inserts of the deposited clone pool.

More precisely, the nucleotide sequence encoding the FRADJ and/or CRYPTIC polypeptide or a fragment or variant thereof is fused to a polynucleotide encoding the DNA binding domain of the GAL4 protein, the fused nucleotide sequence being inserted in a suitable expression vector, for example pAS2 or pM3.

Then, a human cDNA library is constructed in a specially designed vector, such that the human cDNA insert is fused to a nucleotide sequence in the vector that encodes the transcriptional domain of the GAL4 protein. Preferably, the vector used is the pACT vector. The polypeptides encoded by the nucleotide inserts of the human cDNA library are termed "prey" polypeptides.

A third vector contains a detectable marker gene, such as beta galactosidase gene or CAT gene that is placed under the control of a regulation sequence that is responsive to the binding of a complete Gal4 protein containing both the transcriptional activation domain and the DNA binding domain. For example, the vector pG5EC may be used.

Two different yeast strains are also used. As an illustrative but non-limiting example the two different yeast strains may be the following:

Y190, the phenotype of which is (MATa, Leu2-3, 112 ura3-12, trp1-901, his3-D200, ade2-101, gal4Dgal180D URA3 GAL-LacZ, LYS GAL-HIS3, cyh^r);

Y187, the phenotype of which is (MATa gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3, -112 URA3 GAL-lacZmet$^-$), which is the opposite mating type of Y190.

Briefly, 20 µg of pAS2/FRADJ and/or CRYPTIC and 20 µg of pACT-cDNA library are co-transformed into yeast strain Y190. The transformants are selected for growth on minimal media lacking histidine, leucine and tryptophan, but containing the histidine synthesis inhibitor 3-AT (50 mM). Positive colonies are screened for beta galactosidase by filter lift assay. The double positive colonies (His^+, beta-gal^+) are then grown on plates lacking histidine, leucine, but containing tryptophan and

cyclohexamide (10 mg/ml) to select for loss of pAS2/FRADJ and/or CRYPTIC plasmids but retention of pACT-cDNA library plasmids. The resulting Y190 strains are mated with Y187 strains expressing FRADJ and/or CRYPTIC or non-related control proteins; such as cyclophilin B, lamin, or SNF1, as Gal4 fusions as described by Harper *et al.* (1993) and by Bram *et al.* (1993), which disclosures are hereby incorporated by reference in their entireties, and screened for beta galactosidase by filter lift assay. Yeast clones that are beta gal- after mating with the control Gal4 fusions are considered false positives.

In another embodiment of the two-hybrid method according to the invention, interaction between the FRADJ and/or CRYPTIC or a fragment or variant thereof with cellular proteins may be assessed using the Matchmaker Two Hybrid System 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the kit, the disclosure of which is incorporated herein by reference, nucleic acids encoding the FRADJ and/or CRYPTIC protein or a portion thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. A desired cDNA, preferably human cDNA, is inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells that are positive in both the histidine selection and the lacZ assay contain interaction between FRADJ and/or CRYPTIC and the protein or peptide encoded by the initially selected cDNA insert.

Compounds Modulating FRADJ and/or CRYPTIC biological activity

Another method of screening for compounds that modulate gene expression and/or biological activity is by measuring the effects of test compounds on specific biological activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described herein, in a host cell. In one embodiment, the present invention relates to a method of identifying an agent that alters gene activity, wherein a nucleic acid construct comprising a nucleic acid that encodes a mammalian gene polypeptide is introduced into a host cell. The host cells produced are maintained under conditions appropriate for expression of the encoded mammalian gene polypeptides, whereby the nucleic acid is expressed. The host cells are then contacted with a compound to be assessed (an agent) and an activity of the cells is detected in the presence of the compound to be assessed, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described herein. Detection of a change in said activity in the presence of the agent indicates that the agent alters FRADJ and/or CRYPTIC activity. In a particular embodiment, the invention relates to a method of identifying an agent which is an activator of FRADJ and/or CRYPTIC activity, wherein detection of an increase of said activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described

herein, in the presence of the agent indicates that the agent activates FRADJ and/or CRYPTIC activity. In another particular embodiment, the invention relates to a method of identifying an agent which is an inhibitor of FRADJ and/or CRYPTIC activity, wherein detection of a decrease of said activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described herein, in the presence of the agent indicates that the agent inhibits FRADJ and/or CRYPTIC activity.

Detection of a change in said FRADJ and/or CRYPTIC activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described herein, can be performed using a variety of techniques as described for representative activities in examples provided herein. For example, said activity can be used in conjunction with recombinant cells expressing a FRADJ and/or CRYPTIC polypeptide. Decrease of said activity in the presence of the test compound, indicates a decrease of FRADJ and/or CRYPTIC activity, said activity being selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity or as described herein, and increase of said activity in the presence of the test compound, indicates an increase for cases FRADJ and/or CRYPTIC activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. If a decrease in said activity is observed in FRADJ and/or CRYPTIC expressing cells, but not in control cells, then the test compound is an inhibitor of FRADJ and/or CRYPTIC. If an increase in said activity is observed in FRADJ and/or CRYPTIC expressing cells, but not in control cells, then the test compound is an activator of FRADJ and/or CRYPTIC.

In a particular embodiment a high throughput screen can be used to identify agents that activate (enhance) or inhibit FRADJ and/or CRYPTIC activity (See e.g., PCT publication WO 98/45438, which disclosure is hereby incorporated by reference in its entirety). For example, the method of identifying an agent that alters FRADJ and/or CRYPTIC activity can be performed as follows. A nucleic acid construct comprising polynucleotide that encodes a mammalia FRADJ and/or CRYPTIC polypeptide is introduced into a host cell to produce recombinant host cells. The recombinant host cells produced are maintained under conditions appropriate for expression of the encoded mammalia FRADJ and/or CRYPTIC polypeptide, whereby the nucleic acid is expressed. An indicator of FRADJ and/or CRYPTIC activity and the compound to be assessed are added to the recombinant host cells; the resulting combination is referred to as a test sample. The indicator signal is detected. A decrease of indicator signal in the presence of the test compound occurs with a decrease in the FRADJ and/or CRYPTIC activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity of the cells, which indicates that the agent is an inhibitor of FRADJ and/or CRYPTIC. Conversely, an increase of indicator signal in the presence of the test compound occurs with an increase in the FRADJ and/or CRYPTIC activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity of the cells, which indicates that the agent is

an activator of FRADJ and/or CRYPTIC. A control can be used in the methods of detecting agents that alter FRADJ and/or CRYPTIC activity. For example, the control sample includes the same reagents but lacks the compound or agent being assessed; it is treated in the same manner as the test sample.

Methods of Screening for Compounds Modulating FRADJ and/or CRYPTIC Expression and/or Activity

The present invention also relates to methods of screening compounds for their ability to modulate (e.g. increase or inhibit) the activity or expression of FRADJ and/or CRYPTIC. More specifically, the present invention relates to methods of testing compounds for their ability either to increase or to decrease expression or activity of FRADJ and/or CRYPTIC. The assays are performed *in vitro* or *in vivo.*

In vitro methods

In vitro, cells expressing FRADJ and/or CRYPTIC are incubated in the presence and absence of the test compound. By determining the level of FRADJ and/or CRYPTIC expression in the presence of the test compound or the level of FRADJ and/or CRYPTIC activity in the presence of the test compound, compounds can be identified that suppress or enhance FRADJ and/or CRYPTIC expression or activity. Alternatively, constructs comprising a FRADJ and/or CRYPTIC regulatory sequence operably linked to a reporter gene (e.g. luciferase, chloramphenicol acetyl transferase, La cZ, green fluorescent protein, etc.) can be introduced into host cells and the effect of the test compounds on expression of the reporter gene detected. Cells suitable for use in the foregoing assays include, but are not limited to, cells having the same origin as tissues or cell lines in which the polypeptide is known to be expressed.

Consequently, the present invention encompasses a method for screening molecules that modulate the expression of a FRADJ and/or CRYPTIC gene, said screening method comprising the steps of:

a) cultivating a prokaryotic or an eukaryotic cell that has been transfected with a nucleotide sequence encoding a FRADJ and/or CRYPTIC protein or a variant or a fragment thereof, placed under the control of its own promoter;

b) bringing into contact said cultivated cell with a molecule to be tested;

c) quantifying the expression of said FRADJ and/or CRYPTIC protein or a variant or a fragment thereof in the presence of said molecule.

Using DNA recombination techniques well known by the one skill in the art, the FRADJ and/or CRYPTIC protein encoding DNA sequence is inserted into an expression vector, downstream from its promoter sequence. As an illustrative example, the promoter sequence of the FRADJ and/or CRYPTIC gene is contained in the 5' untranscribed region of the FRADJ and/or CRYPTIC genomic DNA.

The quantification of the expression of a FRADJ and/or CRYPTIC protein may be realized either at the mRNA level (using for example Northen blots, RT-PCR, preferably quantitative RT-PCR with primers and probes specific for the FRADJ and/or CRYPTIC mRNA of interest) or at the protein level (using polyclonal or monoclonal antibodies in immunoassays such as ELISA or RIA assays, Western blots, or immunochemistry).

The present invention also concerns a method for screening substances or molecules that are able to increase, or in contrast to decrease, the level of expression of a FRADJ and/or CRYPTIC gene. Such a method may allow the one skilled in the art to select substances exerting a regulating effect on the expression level of a FRADJ and/or CRYPTIC gene and which may be useful as active ingredients included in pharmaceutical compositions for treating patients suffering from diseases/disorders associated with abnormal levels of FRADJ and/or CRYPTIC products, including but not restricted to obesity-related diseases/disorders and disorders associated with excessive weight loss. Obesity and obesity-related diseases and disorders include, but are not restricted to, obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications include, but are not restricted to, microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders include, but are not restricted to, hyperlipidemia and hyperuricemia. Disorders associated with excessive weight loss include, but are not restricted to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

Thus, also part of the present invention is a method for screening a candidate molecule that modulates the expression of a FRADJ and/or CRYPTIC gene, this method comprises the following steps:

a) providing a recombinant cell host containing a nucleic acid, wherein said nucleic acid comprises a FRADJ and/or CRYPTIC 5' regulatory region or a regulatory active fragment or variant thereof, operably linked to a polynucleotide encoding a detectable protein;

b) obtaining a candidate molecule; and

c) determining the ability of said candidate molecule to modulate the expression levels of said polynucleotide encoding the detectable protein.

In a further embodiment, said nucleic acid comprising a FRADJ and/or CRYPTIC 5' regulatory region or a regulatory active fragment or variant thereof, includes the 5'UTR region of a FRADJ and/or CRYPTIC cDNA selected from the group comprising of the 5'UTRs of the sequences of SEQ ID Nos 1, 3, sequences of clones inserts of the deposited clone pool, regulatory active fragments and variants thereof. In a more preferred embodiment of the above screening method, said nucleic acid includes a promoter sequence which is endogenous with respect to the FRADJ and/or CRYPTIC 5'UTR sequence. In another more preferred embodiment of the above screening method, said nucleic acid includes a promoter sequence which is exogenous with respect to the FRADJ and/or CRYPTIC 5'UTR sequence defined therein.

Preferred polynucleotides encoding a detectable protein are polynucleotides encoding beta galactosidase, green fluorescent protein (GFP) and chloramphenicol acetyl transferase (CAT).

The invention further relates to a method for the production of a pharmaceutical composition comprising a method of screening a candidate molecule that modulates the expression of a FRADJ and/or CRYPTIC gene and furthermore mixing the identified molecule with a pharmaceutically acceptable carrier.

The invention also pertains to kits for the screening of a candidate substance modulating the expression of a FRADJ and/or CRYPTIC gene. Preferably, such kits comprise a recombinant vector that allows the expression of a FRADJ and/or CRYPTIC 5' regulatory region or a regulatory active fragment or a variant thereof, operably linked to a polynucleotide encoding a detectable protein or a FRADJ and/or CRYPTIC protein or a fragment or a variant thereof. More preferably, such kits include a recombinant vector that comprises a nucleic acid including the 5'UTR region of a FRADJ and/or CRYPTIC cDNA selected from the group comprising the 5'UTRs of the sequences of SEQ ID No 1 or 3, sequences of clones inserts of the deposited clone pool, regulatory active fragments and variants thereof, being operably linked to a polynucleotide encoding a detectable protein.

For the design of suitable recombinant vectors useful for performing the screening methods described above, it will be referred to the section of the present specification wherein the preferred recombinant vectors of the invention are detailed.

Another object of the present invention comprises methods and kits for the screening of candidate substances that interact with a FRADJ and/or CRYPTIC polypeptide, fragments or variants thereof. By their capacity to bind covalently or non-covalently to a FRADJ and/or CRYPTIC protein, fragments or variants thereof, these substances or molecules may be advantageously used both *in vitro* and *in vivo*.

In vitro, said interacting molecules may be used as detection means in order to identify the presence of a FRADJ and/or CRYPTIC protein in a sample, preferably a biological sample.

A method for the screening of a candidate substance that interact with a FRADJ and/or CRYPTIC polypeptide, fragments or variants thereof, said methods comprising the following steps:

a) providing a polypeptide comprising, consisting essentially of, or consisting of a FRADJ and/or CRYPTIC protein or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 and 4 and polypeptides encoded by the clone inserts of the deposited clone pool;

b) obtaining a candidate substance;

c) bringing into contact said polypeptide with said candidate substance;

d) detecting the complexes formed between said polypeptide and said candidate substance.

The invention further relates to a method for the production of a pharmaceutical composition comprising a method for the screening of a candidate substance that interact with a FRADJ and/or CRYPTIC polypeptide, fragments or variants thereof and furthermore mixing the identified substance with a pharmaceutically acceptable carrier.

The invention further concerns a kit for the screening of a candidate substance interacting with the FRADJ and/or CRYPTIC polypeptide, wherein said kit comprises:

a) a polypeptide comprising, consisting essentially of, or consisting of a FRADJ and/or CRYPTIC protein or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO:2 and 4 and polypeptides encoded by the clone inserts of the deposited clone pool; and

b) optionally means useful to detect the complex formed between said polypeptide or a variant thereof and the candidate substance.

In a preferred embodiment of the kit described above, the detection means comprises a monoclonal or polyclonal antibody binding to said FRADJ and/or CRYPTIC protein or fragment or variant thereof.

In vivo methods

Compounds that suppress or enhance FRADJ and/or CRYPTIC expression can also be identified using *in vivo* screens. In these assays, the test compound is administered (e.g. IV, IP, IM, orally, or otherwise), to the animal, for example, at a variety of dose levels. The effect of the compound on FRADJ and/or CRYPTIC expression is determined by comparing FRADJ and/or CRYPTIC levels, for

example in tissues known to express the gene of interest, and using Northern blots, immunoassays, PCR, etc., as described above. Suitable test animals include rodents (e.g., mice and rats), primates. Humanized mice can also be used as test animals, that is mice in which the endogenous mouse protein is ablated (knocked out) and the homologous human protein added back by standard transgenic approaches. Such mice express only the human form of a protein. Humanized mice expressing only the huma FRADJ and/or CRYPTIC can be used to study *in vivo* responses of obesity-related diseases/disorders and disorders associated with esxcessive weight loss in response to potential agents regulating FRADJ and/or CRYPTIC protein or mRNA levels. As an example, transgenic mice have been produced carrying the human apoE4 gene. They are then bred with a mouse line that lacks endogenous apoE, to produce an animal model carrying human proteins believed to be instrumental in development of Alzheimer's pathology. Such transgenic animals are useful for dissecting the biochemical and physiological steps of disease, and for development of therapies for disease intervention (Loring, *et al*, 1996) (incorporated herein by reference in its entirety).

Uses for compounds modulating FRADJ and/or CRYPTIC expression and/or biological activity

Using *in vivo* (or *in vitro)* systems, it may be possible to identify compounds that exert a tissue specific effect, for example, that increase FRADJ and/or CRYPTIC expression or activity only in tissues of interest, such as muscle, liver, blood, and adipose tissue. Screening procedures such as those described above are also useful for identifying agents for their potential use in pharmacological intervention strategies. Agents that enhance FRADJ and/or CRYPTIC expression or stimulate its activity may thus be used to induce associated, for example, with obesity-related diseases/disorders or disorders associated with excessive weight loss. Obesity-related diseases and disorders such as obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

In addition, agents that enhance FRADJ and/or CRYPTIC expression or activity may also be used to treat disorders such as other obesity-related diseases/disorders. Other obesity-related disorders include hyperlipidemia and hyperuricemia.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

Compounds that suppress FRADJ and/or CRYPTIC expression or inhibit its activity can be used to treat disorders associated with excessive weight loss. Disorders associated with excessive weight loss include, but are not limited to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

In addition, agents that suppress FRADJ and/or CRYPTIC expression or inhibit its activity may also be used to treat disorders such as disorders associated with excessive weight loss. Disorders associates with excessive weight loss include, but are not limited to, cachexia, wasting, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

Also encompassed by the present invention is an agent that interacts with FRADJ and/or CRYPTIC directly or indirectly, and inhibits or enhances FRADJ and/or CRYPTIC expression and/or function. In one embodiment, the agent is an inhibitor that interferes with FRADJ and/or CRYPTIC directly (e.g., by binding FRADJ and/or CRYPTIC) or indirectly (e.g., by blocking the ability of FRADJ and/or CRYPTIC to have a FRADJ and/or CRYPTIC biological activity). In a particular embodiment, an inhibitor of FRADJ and/or CRYPTIC protein is an antibody specific for FRADJ and/or CRYPTIC protein or a functional portion of FRADJ and/or CRYPTIC; that is, the antibody binds a FRADJ and/or CRYPTIC polypeptide. For example, the antibody can be specific for a polypeptide encoded by one of the amino acid sequences of human genes (SEQ ID NO:2 and 4), mammal FRADJ and/or CRYPTIC or portions thereof. Alternatively, the inhibitor can be an agent other than an antibody (e.g., small organic molecule, protein or peptide) that binds FRADJ and/or CRYPTIC and blocks its activity. For example, the inhibitor can be an agent that mimics FRADJ and/or CRYPTIC structurally, but lacks its function. Alternatively, it can be an agent that binds to or interacts with a molecule that FRADJ and/or CRYPTIC normally binds with or interacts with, thus blocking FRADJ and/or CRYPTIC from doing so and preventing it from exerting the effects it would normally exert.

In another embodiment, the agent is an enhancer (activator) of FRADJ and/or CRYPTIC that increases the activity of FRADJ and/or CRYPTIC (increases the effect of a given amount or level of FRADJ and/or CRYPTIC), increases the length of time it is effective (by preventing its degradation or otherwise prolonging the time during which it is active) or both either directly or indirectly. For example, FRADJ and/or CRYPTIC polynucleotides and polypeptides can be used to identify therapeutic drugs.

The FRADJ and/or CRYPTIC sequences of the present invention can also be used to generate nonhuman gene knockout animals, such as mice, which lack a FRADJ and/or CRYPTIC gene or transgenically overexpress FRADJ and/or CRYPTIC. For example, such FRADJ and/or CRYPTIC gene knockout mice can be generated and used to obtain further insight into the function of FRADJ and/or CRYPTIC as well as assess the specificity of FRADJ and/or CRYPTIC activators and inhibitors. Also, over expression of FRADJ and/or CRYPTIC (e.g., huma FRADJ and/or CRYPTIC) in transgenic mice can be used as a means of creating a test system for FRADJ and/or CRYPTIC activators and inhibitors (e.g., against huma FRADJ and/or CRYPTIC). In addition, the FRADJ and/or CRYPTIC gene can be used to clone the FRADJ and/or CRYPTIC promoter/enhancer in order to identify regulators of FRADJ and/or CRYPTIC transcription. FRADJ and/or CRYPTIC gene knockout animals include animals that completely or partially lack the FRADJ and/or CRYPTIC gene and/or FRADJ and/or CRYPTIC a ctivity or function. As described herein, it is likely that FRADJ and/or CRYPTIC plays a role in obesity-related diseases/disorders and disorders associated with excessive weight loss, which indicates that inhibitors of FRADJ and/or CRYPTIC can be used as a means of said diseases/disorders. Thus the present invention relates to a method of inhibiting (partially or completely) FRADJ and/or CRYPTIC biological function in a mammal (e.g., human) comprising administering to the mammal an effective amount of an inhibitor of FRADJ and/or CRYPTIC. The invention also relates to a method of enhancing FRADJ and/or CRYPTIC biological function in a mammal comprising administering to the mammal an effective amount of an enhancer FRADJ and/or CRYPTIC.

Inhibiting FRADJ and/or CRYPTIC expression

Therapeutic compositions according to the present invention may comprise advantageously one or several FRADJ and/or CRYPTIC oligonucleotide fragments as an antisense tool or a triple helix tool that inhibits the expression of the corresponding FRADJ and/or CRYPTIC gene.

Antisense Approach

In antisense approaches, nucleic acid sequences complementary to an mRNA are hybridized to the mRNA intracellularly, thereby blocking the expression of the protein encoded by the mRNA. The antisense nucleic acid molecules to be used in gene therapy may be either DNA or RNA sequences. Preferred methods using antisense polynucleotide according to the present invention are the procedures described by Sczakiel *et al.* (1995), which disclosure is hereby incorporated by reference in its entirety.

Preferably, the antisense tools are chosen among the polynucleotides (15 -200 bp long) that are complementary to FRADJ and/or CRYPTIC mRNA, more preferably to the 5'end of the FRADJ and/or CRYPTIC mRNA. In another embodiment, a combination of different antisense polynucleotides complementary to different parts of the desired targeted gene are used.

Other preferred antisense polynucleotides according to the present invention are sequences complementary to either a sequence of FRADJ and/or CRYPTIC mRNAs comprising the translation initiation codon ATG or a sequence of FRADJ and/or CRYPTIC genomic DNA containing a splicing donor or acceptor site.

Preferably, the antisense polynucleotides of the invention have a 3' polyadenylation signal that has been replaced with a self-cleaving ribozyme sequence, such that RNA polymerase II transcripts are produced without poly(A) at their 3' ends, these antisense polynucleotides being incapable of export from the nucleus, such as described by Liu *et al.* (1994), which disclosure is hereby incorporated by reference in its entirety. In a preferred embodiment, these FRADJ and/or CRYPTIC antisense polynucleotides also comprise, within the ribozyme cassette, a histone stem-loop structure to stabilize cleaved transcripts against 3'-5' exonucleolytic degradation, such as the structure described by Eckner *et al.* (1991), which disclosure is hereby incorporated by reference in its entirety.

The antisense nucleic acids should have a length and melting temperature sufficient to permit formation of an intracellular duplex having sufficient stability to inhibit the expression of the FRADJ and/or CRYPTIC mRNA in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green *et al.*, (1986) and Izant and Weintraub, (1984), the disclosures of which are incorporated herein by reference.

In some strategies, antisense molecules are obtained by reversing the orientation of the FRADJ and/or CRYPTIC coding region with respect to a promoter so as to transcribe the opposite strand from that which is normally transcribed in the cell. The antisense molecules may be transcribed using *in vitro* transcription systems such as those which employ T7 or SP6 polymerase to generate the transcript. Another approach involves transcription of FRADJ and/or CRYPTIC antisense nucleic acids *in vivo* by operably linking DNA containing the antisense sequence to a promoter in a suitable expression vector.

Alternatively, oligonucleotides that are complementary to the strand normally transcribed in the cell may be synthesized *in vitro*. Thus, the antisense nucleic acids are complementary to the corresponding mRNA and are capable of hybridizing to the mRNA to create a duplex. In some embodiments, the antisense sequences may contain modified sugar phosphate backbones to increase stability and make them less sensitive to RNase activity. Examples of modifications suitable for use in antisense strategies include 2' O-methyl RNA oligonucleotides and Protein-nucleic acid (PNA) oligonucleotides. Further examples are described by Rossi *et al.*, (1991), which disclosure is hereby incorporated by reference in its entirety.

Various types of antisense oligonucleotides complementary to the sequence of the FRADJ and/or CRYPTIC cDNA or genomic DNA may be used. In one preferred embodiment, stable and semi-stable antisense oligonucleotides described in International Application No. PCT WO94/23026, hereby incorporated by reference, are used. In these molecules, the 3' end or both the 3' and 5' ends are engaged in intramolecular hydrogen bonding between complementary base pairs. These molecules are better able to withstand exonuclease attacks and exhibit increased stability compared to conventional antisense oligonucleotides.

In another preferred embodiment, the antisense oligodeoxynucleotides against herpes simplex virus types 1 and 2 described in International Application No. WO 95/04141, hereby incorporated by reference, are used.

In yet another preferred embodiment, the covalently cross-linked antisense oligonucleotides described in International Application No. WO 96/31523, hereby incorporated by reference, are used. These double- or single-stranded oligonucleotides comprise one or more, respectively, inter- or intra-oligonucleotide covalent cross-linkages, wherein the linkage consists of an amide bond between a primary amine group of one strand and a carboxyl group of the other strand or of the same strand, respectively, the primary amine group being directly substituted in the 2' position of the strand nucleotide monosaccharide ring, and the carboxyl group being carried by an aliphatic spacer group substituted on a nucleotide or nucleotide analog of the other strand or the same strand, respectively.

The antisense oligodeoxynucleotides and oligonucleotides disclosed in International Application No. WO 92/18522, incorporated by reference, may also be used. These molecules are stable to degradation and contain at least one transcription control recognition sequence that binds to control proteins and are effective as decoys therefore. These molecules may contain "hairpin" structures, "dumbbell" structures, "modified dumbbell" structures, "cross-linked" decoy structures and "loop" structures.

In another preferred embodiment, the cyclic double-stranded oligonucleotides described in European Patent Application No. 0 572 287 A2, hereby incorporated by reference are used. These ligated oligonucleotide "dumbbells" contain the binding site for a transcription factor and inhibit expression of the gene under control of the transcription factor by sequestering the factor.

Use of the closed antisense oligonucleotides disclosed in International Application No. WO 92/19732, hereby incorporated by reference, is also contemplated. Because these molecules have no free ends, they are more resistant to degradation by exonucleases than are conventional oligonucleotides. These oligonucleotides may be multifunctional, interacting with several regions which are not adjacent to the target mRNA.

The appropriate level of antisense nucleic acids required to inhibit gene expression may be determined using *in vitro* expression analysis. The antisense molecule may be introduced into the cells

by diffusion, injection, infection or transfection using procedures known in the art. For example, the antisense nucleic acids can be introduced into the body as a bare or naked oligonucleotide, oligonucleotide encapsulated in lipid, oligonucleotide sequence encapsidated by viral protein, or as an oligonucleotide operably linked to a promoter contained in an expression vector. The expression vector may be any of a variety of expression vectors known in the art, including retroviral or viral vectors, vectors capable of extrachromosomal replication, or integrating vectors. The vectors may be DNA or RNA.

The antisense molecules are introduced onto cell samples at a number of different concentrations preferably between $1x10^{-10}$M to $1x10^{-4}$M. Once the minimum concentration that can adequately control gene expression is identified, the optimized dose is translated into a dosage suitable for use *in vivo*. For example, an inhibiting concentration in culture of $1x10^{-7}$ translates into a dose of approximately 0.6 mg/kg bodyweight. Levels of oligonucleotide approaching 100 mg/kg bodyweight or higher may be possible after testing the toxicity of the oligonucleotide in laboratory animals. It is additionally contemplated that cells from the vertebrate are removed, treated with the antisense oligo nucleotide, and reintroduced into the vertebrate.

In a preferred application of this invention, the polypeptide encoded by the gene is first identified, so that the effectiveness of antisense inhibition on translation can be monitored using techniques that include but are not limited to antibody-mediated tests such as RIAs and ELISA, functional assays, or radiolabeling.

An alternative to the antisense technology that is used according to the present invention comprises using ribozymes that will bind to a target sequence via their complementary polynucleotide tail and that will cleave the corresponding RNA by hydrolyzing its target site (namely "hammerhead ribozymes"). Briefly, the simplified cycle of a hammerhead ribozyme comprises: (1) sequence specific binding to the target RNA via complementary antisense sequences; (2) site-specific hydrolysis of the cleavable motif of the target strand; and (3) release of cleavage products, which gives rise to another catalytic cycle. Indeed, the use of long-chain antisense polynucleotide (at least 30 bases long) or ribozymes with long antisense arms are advantageous. A preferred delivery system for antisense ribozyme is achieved by covalently linking these antisense ribozymes to lipophilic groups or to use liposomes as a convenient vector. Preferred antisense ribozymes according to the present invention are prepared as described by Rossi *et al*, (1991) and Sczakiel *et al.* (1995), the specific preparation procedures being referred to in said articles being herein incorporated by reference.

Triple Helix Approach

The FRADJ and/or CRYPTIC genomic DNA may also be used to inhibit the expression of the FRADJ and/or CRYPTIC gene based on intracellular triple helix formation.

Triple helix oligonucleotides are used to inhibit transcription from a genome. They are particularly useful for studying alterations in cell activity when it is associated with a particular gene. The FRADJ and/or CRYPTIC cDNA or genomic DNAs of the present invention or, more preferably, a fragment of those sequences, can be used to inhibit gene expression in individuals having diseases associated with expression of a particular gene. Similarly, a portion of the FRADJ and/or CRYPTIC genomic DNA can be used to study the effect of inhibiting FRADJ and/or CRYPTIC transcription within a cell. Traditionally, homopurine sequences were considered the most useful for triple helix strategies. However, homopyrimidine sequences can also inhibit gene expression. Such homopyrimidine oligonucleotides bind to the major groove at homopurine:homopyrimidine sequences. Thus, both types of sequences from the FRADJ and/or CRYPTIC genomic DNA are contemplated within the scope of this invention.

To carry out gene therapy strategies using the triple helix approach, the sequences of the FRADJ and/or CRYPTIC genomic DNA are first scanned to identify 10-mer to 20-mer homopyrimidine or homopurine stretches which could be used in triple-helix based strategies for inhibiting FRADJ and/or CRYPTIC expression. Following identification of candidate homopyrimidine or homopurine stretches, their efficiency in inhibiting FRADJ and/or CRYPTIC expression is assessed by introducing varying amounts of oligonucleotides containing the candidate sequences into tissue culture cells which express the FRADJ and/or CRYPTIC gene.

The oligonucleotides can be introduced into the cells using a variety of methods known to those skilled in the art, including but not limited to calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection or native uptake.

Treated cells are monitored for altered cell function or reduced FRADJ and/or CRYPTIC expression using techniques such as Northern blotting, RNase protection assays, or PCR based strategies to monitor the transcription levels of the FRADJ and/or CRYPTIC gene in cells which have been treated with the oligonucleotide. The cell functions to be monitored are predicted based upon the homologies of the target gene corresponding to the cDNA from which the oligonucleotide was derived with known gene sequences that have been associated with a particular function. The cell functions can also be predicted based on the presence of abnormal physiology within cells derived from individuals with a particular inherited disease, particularly when the cDNA is associated with the disease using techniques described in the section entitled "Identification of genes associated with hereditary diseases or drug response".

The oligonucleotides which are effective in inhibiting gene expression in tissue culture cells may then be introduced *in vivo* using the techniques and at a dosage calculated based on the *in vitro* results, as described in the section entitled "Antisense Approach".

In some embodiments, the natural (beta) anomers of the oligonucleotide units can be replaced with alpha anomers to render the oligonucleotide more resistant to nucleases. Further, an intercalating

agent such as ethidium bromide, or the like, can be attached to the 3' end of the alpha oligonucleotide to stabilize the triple helix. For information on the generation of oligonucleotides suitable for triple helix formation see Griffin *et al.* (1989), which is hereby incorporated by this reference.

Treating FRADJ and/or CRYPTIC-related disorders

The present invention further relates to methods of treating diseases/disorders including but not restricted to obesity-related diseases/disorders and disorders associated with excessive weight loss. Obesity-related diseases and disorders include, but are not restricted to, obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Noninsulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications include, but are not restricted to, microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders include, but are not restricted to, hyperlipidemia and hyperuricemia. Disorders associated with excessive weight loss include, but are not restricted to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia. In highly preferred embodiments, the pharmaceutical compositions are used to modulate body weight for cosmetic reasons.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, alone, without combination of insulin therapy.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some individuals, particularly those with Type II diabetes or insulin resistance, alone, without combination of insulin therapy.

In a further preferred embodiment, AGONISTS of the present invention may be used in complementary therapy, particularly in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, to improve their weight or glucose control in combination with an insulin secretagogue or an insulin sensitising agent. Preferably, the insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl)guanidine fumarate (BTS67582) or a sulphonylurea selected from tolbutamide, tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

AGONISTS of the present invention further provide a method of improving the body weight or glucose control of some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, AGONISTS of the present invention may be administered either concomitantly or concurrently, with the insulin secretagogue or insulin sensitising agent for example in the form of separate dosage units to be used simultaneously, separately or sequentially (either before or after the secretagogue or either before or after the sensitising agent). Accordingly, the present invention further provides for a composition of pharmaceutical or physiologically acceptable composition and an insulin secretagogue or insulin sensitising agent as a combined preparation for simultaneous, separate or sequential use for the improvement of body weight or glucose control in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an insulin sensitiser.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, AGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some individuals, particularly those with Type II diabetes or insulin resistance, without insulin therapy.

In further preferred embodiments, ANTAGONISTS of the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to treat excessive weight loss in some individuals, particularly those with, but not restricted to, cachexia, wasting, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, anorexia, and bulimia.

The FRADJ and/or CRYPTIC or LIGAND polypeptide fragments or ANTAGONISTS thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

These methodologies can be effected using compounds selected using screening protocols such as those described herein and/or by using the gene therapy and antisense approaches described in the art and herein. Gene therapy can be used to effect targeted expression of FRADJ and/or CRYPTIC. The FRADJ and/or CRYPTIC coding sequence can be cloned into an appropriate expression vector and targeted to a particular cell type(s) to achieve efficient, high level expression. Introduction of the FRADJ and/or CRYPTIC coding sequence into target cells can be achieved, for example, using particle mediated DNA delivery, (Haynes, 1996 and Maurer, 1999), direct injection of naked DNA, (Levy *et al.*, 1996; and Felgner, 1996), or viral vector mediated transport (Smith *et al.*, 1996, Stone et al, 2000; Wu and Atai, 2000), each of which disclosures are hereby incorporated by reference in their entireties. Tissue specific effects can be achieved, for example, in the case of virus mediated transport by using viral vectors that are tissue specific, or by the use of promoters that are tissue specific.

Combinatorial approaches can also be used to ensure that the FRADJ and/or CRYPTIC coding sequence is activated in the target tissue (Butt and Karathanasis, 1995; Miller and Whelan, 1997), which disclosures are hereby incorporated by reference in their entireties. Antisense oligonucleotides complementary to FRADJ and/or CRYPTIC mRNA can be used to selectively diminish or ablate the expression of the protein, for example, at sites of inflammation. More specifically, antisense constructs or antisense oligonucleotides can be used to inhibit the production of FRADJ and/or CRYPTIC in high expressing cells. Antisense mRNA can be produced by transfecting into target cells an expression vector with the FRADJ and/or CRYPTIC gene sequence, or portion thereof, oriented in an antisense direction relative to the direction of transcription. Appropriate vectors include viral vectors, including retroviral, adenoviral, and adeno-associated viral vectors, as well as nonviral vectors. Tissue specific promoters can be used. Alternatively, antisense oligonucleotides can be introduced directly into target cells to achieve the same goal. (See also other delivery methodologies described herein in connection with FRADJ and/or CRYPTIC therapy.). Oligonucleotides can be selected/designed to achieve a high level of specificity (Wagner *et al.*, 1996), which disclosure is hereby incorporated by reference in its entirety. The therapeutic methodologies described herein are applicable to both human and non-human mammals (including cats and dogs).

Pharmaceutical and physiologically acceptable compositions

The present invention also relates to pharmaceutical or physiologically acceptable compositions comprising, as active agent, the polypeptides, nucleic acids or antibodies of the invention. The invention also relates to compositions comprising, as active agent, compounds selected using the above-described

screening protocols. Such compositions include the active agent in combination with a pharmaceutical or physiologically acceptably acceptable carrier. In the case of naked DNA, the "carrier" may be gold particles. The amount of active agent in the composition can vary with the agent, the patient and the effect sought. Likewise, the dosing regimen can vary depending on the composition and the disease/disorder to be treated.

Therefore, the invention related to methods for the production of pharmaceutical composition comprising a method for selecting an active agent, compound, substance or molecule using any of the screening method described herein and furthermore mixing the identified active agent, compound, substance or molecule with a pharmaceutically acceptable carrier.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co. Easton, Pa).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through a combination of active compounds with solid excipient, sulting mixture is optionally grinding, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums inc luding arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titaniumdioxide, lacquer solutions, and suitable organ ic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquidpolyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethylcellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of FRADJ and/or CRYPTIC, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The

animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example FRADJ and/or CRYPTIC or fragments thereof, antibodies of FRADJ and/or CRYPTIC, AGONISTS, ANTAGONISTS or inhibitors of FRADJ and/or CRYPTIC, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions maybe administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

LIGAND/APM1 levels

Studies have shown that obese people have lower levels of full-length APM1 than non-obese people (Weyer, 2001). The invention envisions treatment of individuals (preferably obese) that have low levels of full-length LIGAND, preferably APM1, with AGONISTS including, but not restricted to, LIGAND or LIGAND polypeptide fragments of the invention. In addition, the invention preferably is drawn to treatment of individuals with low levels of biologically active fragment of said LIGAND, preferably APM1, with AGONISTS including, but not restricted to, LIGAND or LIGAND polypeptide fragments of the invention. In further embodiments, said AGONIST of the present invention is

administered to individuals, preferably obese individuals, that have levels of full-length LIGAND, preferably APM1, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, about 100% or 100% lower than non-obese individuals, preferably healthy individuals as determined by a physician using normal standards in the art. In further embodiments, said AGONIST of the present invention is administered to individuals, preferably obese individuals, that have levels of C-terminal fragment of LIGAND, preferably APM1, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, about 100% or 100% lower than non-obese individuals, preferably healthy individuals as determined by a physician using normal standards in the art.

The invention further envisions treatment of individuals (preferably manifesting excessive weight loss) that have high levels of full-length LIGAND, preferably APM1, with ANTAGONISTS including, but not restricted to, soluble fragment of FRADJ and/or CRYPTIC polypeptide and LIGAND or LIGAND polypeptide fragments of the invention. In addition, the invention preferably is drawn to treatment of individuals with high levels of biologically active fragment of said LIGAND, preferably APM1, with ANTAGONISTS including, but not restricted to, soluble fragment of FRADJ and/or CRYPTIC polypeptide and LIGAND or LIGAND polypeptide fragments of the invention. In further embodiments, said ANTAGONIST of the present invention is administered to individuals, preferably individuals manifesting excessive weight loss, that have levels of full-length LIGAND, preferably APM1, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, about 100% or greater than 100% higher than normal individuals, preferably healthy individuals as determined by a physician using normal standards in the art. In further embodiments, said ANTAGONIST of the present invention is administered to individuals, preferably obese individuals, that have levels of C-terminal fragment of LIGAND, preferably APM1, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, about 100% or greater than 100% higher than normal individuals, preferably healthy individuals as determined by a physician using normal standards in the art. Methods to determine and compare the levels of full-length or fragment of LIGAND, preferably APM1, in individuals are well-known in the art and include, but are not limited to using an antibody specific for APM1 in a format such as a Radio Immune Assay, ELISA, Western blot, dotblot, or as part of an array, for example. Methods of generating antibodies to LIGAND, preferably APM1, and fragments thereof as well as to proteins with SNPs are discussed in PCT/IB99/01858, US application No. 09/434,848, and WO 99/07736, hereby incorporated herein by reference in its entirety including and drawings, figures, or tables. Further, antibodies specific for polypeptide fragments of the invention, their generation, and their use are described herein.

Preparation of Antibody Compositions to the FRADJ and/or CRYPTIC protein

Substantially pure protein or polypeptide is isolated from transfected or transformed cells containing an expression vector encoding the FRADJ and/or CRYPTIC protein or a portion thereof. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon

filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

A. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes in the FRADJ and/or CRYPTIC protein or a portion thereof can be prepared from murine hybridomas according to the classical method of Kohler and Milstein, (1975) or derivative methods thereof. Also see Harlow and Lane. (1988)..

Briefly, a mouse is repetitively inoculated with a few micrograms of the FRADJ and/or CRYPTIC protein or a portion thereof over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, (1980), which disclosure is hereby incorporated by reference in its entirety, and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, *et al.* (1986) Section 21-2.

Also particularly included in the present invention are monoclonal antibodies that specifically bind FRADJ and/or CRYPTIC polypeptide. Preferably the present invention includes monoclonal antibodies that specifically bind FRADJ and/or CRYPTIC polypeptide fragment comprising the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 and 4, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide.

Additionally particularly included in the present invention are monoclonal antibodies that specifically bind LIGAND polypeptide. Further particularly included in the present invention are monoclonal antibodies that specifically bind LIGAND polypeptide fragment. Yet further particularly included in the present invention are monoclonal antibodies that specifically bind APM1 polypeptide fragment comprising amino acids 92-244, 101-244, 108-142, 153-193, 165-176 or 214-227, C2P polypeptide fragment comprising amino acids 181-333, 190-333, 197-233, 244-284, 256-267 or 304-317 or D2P polypeptide fragment comprising amino acids 181-333, 190-333, 197-233, 244-284, 256-267 or 304-317. Most particularly included in the present invention are monoclonal antibodies that specifically bind APM1 polypeptide fragment comprising amino acids 92-244, 101-244, 108-142, 153-193, 165-176 or 214-227. It is understood that said numbering of amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2.

B. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogeneous epitopes in the FRADJ and/or CRYPTIC protein or a portion thereof can be prepared by immunizing suitable non-human animal with the FRADJ and/or CRYPTIC protein or a portion thereof, which can be unmodified or modified to enhance immunogenicity. A suitable non-human animal is preferably a non-human mammal is selected, usually a mouse, rat, rabbit, goat, or horse. Alternatively, a crude preparation that has been enriched for FRADJ and/or CRYPTIC concentration can be used to Generate antibodies. Such proteins, fragments or preparations are introduced into the non-human mammal in the presence of an appropriate adjuvant (e.g. aluminum hydroxide, RIBI, etc.) that is known in the art. In addition the protein, fragment or p reparation can be pretreated with an agent that will increase antigenicity, such agents are known in the art and include, for example, methylated bovine serum albumin (mBSA), bovine serum albumin (BSA), Hepatitis B surface antigen, and keyhole limpet hemocyanin (KLH). Serum from the immunized animal is collected, treated and tested according to known procedures. If the serum contains polyclonal antibodies to undesired epitopes, the polyclonal antibodies can be purified by immunoaffinity chromatography.

Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. Also, host animals vary in response to site of inoculations and dose, with both inadequate and excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appear to be most reliable. Techniques for producing and processing polyclonal antisera are known in the art. An effective immunization protocol for rabbits can be found in Vaitukaitis *et al.* (1971), which disclosure is hereby incorporated by reference in its entirety.

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony *et al.*, (1973), which disclosure is hereby incorporated by reference in its entirety. Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 uM). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher (1980), which disclosure is hereby incorporated by reference in its entirety.

Antibody preparations prepared according to either the monoclonal or the polyclonal protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

Also particularly included in the present invention are polyclonal antibodies that specifically bind FRADJ and/or CRYPTIC polypeptide. Preferably the present invention includes monoclonal antibodies that specifically bind FRADJ and/or CRYPTIC polypeptide fragment comprising the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide.

Additionally particularly included in the present invention are polyclonal antibodies that specifically bind LIGAND polypeptide. Further particularly included in the present invention are polyclonal antibodies that specifically bind LIGAND polypeptide fragment. Yet further particularly included in the present invention are monoclonal antibodies that specifically bind APM1 polypeptide fragment comprising amino acids 92-244, 101-244, 108-142, 153-193, 165-176 or 214-227, C2P polypeptide fragment comprising amino acids 181-333, 190-333, 197-233, 244-284, 256-267 or 304-317 or D2P polypeptide fragment comprising amino acids 181-333, 190-333, 197-233, 244-284, 256-267 or 304-317. Most particularly included in the present invention are monoclonal antibodies that specifically bind APM1 polypeptide fragment comprising amino acids 92-244, 101-244, 108-142, 153-193, 165-176 or 214-227. It is understood that said numbering of amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2.

Assays for Identifying FRADJ and/or CRYPTIC ANTAGONISTS and AGONISTS as Modulators of LIGAND Activity

The invention features methods of screening for one or more compounds that modulate LIGAND or LIGAND polypeptide fragment activity in cells, that includes providing potential compounds to be tested to the cells, and where modulation of a LIGAND or LIGAND polypeptide fragment effect or activity indicates the one or more compounds. Exemplary assays that may be used, wherein the exemplary LIGAND is ACRP30 and the exemplary LIGAND polypeptide fragment is gACRP30 polypeptide fragment, are described in the Examples 4-5, 7-15, 17. ACRP30 is the mouse protein homologue to the human APM1 protein. The globular head form is indicated by placing a 'g' in front, *i.e.* gACRP30. By "gACRP30 polypeptide fragment" is intended a polypeptide fragment of ACRP30, wherein said fragment is comprised of the C-terminal C1q homology globular domain of full length ACRP30. Furthermore, said gACRP30 polypeptide fragment is understood herein to include, but not be restricted to, gACRP30 polypeptide fragment generated as described herein either by enzymatic cleavage of full length ACRP30 or by recombinant DNA methodology. It is further understood that ACRP30 is intended to encompass LIGANDS, wherein said LIGAND is selected from the group of polypeptides comprising Table 2. Preferred LIGAND is APM1. It is further understood that gACRP30 polypeptide

fragment is intended to encompass LIGAND polypeptide fragments, wherein said LIGAND is selected from the group of polypeptides comprising Table 2. Preferred LIGAND polypeptide fr agment is gAPM1 polypeptide fragment.

To these assays would be added compounds to be tested for their inhibitory or stimulatory activity as compared to the effects of ACRP30 polypeptide fragment alone. Other assays in which an effect is observed based on the addition of ACRP30 polypeptide fragment can also be used to screen for modulators of ACRP30 polypeptide fragment activity or effects of the presence of ACRP30 polypeptide fragment on cells. The essential step is to apply an unknown compound and then t o monitor an assay for a change from what is seen when only ACRP30 polypeptide fragment is applied to the cell. A change is defined as something that is significantly different in the presence of the compound plus ACRP30 polypeptide fragment compared to ACRP30 polypeptide fragment alone. In this case, significantly different would be an "increase" or a "decrease" in a measurable effect of at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

The term "modulation" as used herein refers to a measurable change in an activity. Examples include, but are not limited to, lipolysis stimulated receptor (LSR) modulation, leptin modulation, lipoprotein modulation, plasma FFA levels, FFA oxidation, TG levels, glucose levels, and weight. These effects can be *in vitro* or preferably *in vivo*. Modulation of an activity can be either an increase or a decrease in the activity. Thus, LSR activity can be increased or decreased, leptin activity can be increased or decreased, and lipoprotein activity can be increased or decreased. Similarly, FFA, TG, and glucose levels (and weight) can be increased or decreased *in vivo*. Free Fatty Acid oxidation can be increased or decreased *in vivo* or *ex vivo*.

By "LSR" activity is meant expression of LSR on the surface of the cell, or in a particular conformation, as well as its ability to bind, uptake, and degrade leptin and lipoprotein. By "leptin" activity is meant its binding, uptake and degradation by LSR, as well as its transport across a blood brain barrier, and potentially these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Similarly, by "lipoprotein" activity is meant its binding, uptake and degradation by LSR, as well as these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Exemplary assays are provided in Examples 4-5, 7-15, 17, wherein the exemplary LIGAND is ACRP30 and the exemplary LIGAND polypeptide fragment is gACRP30 polypeptide fragment. These assay and other comparabl e assays can be used to determine/identify compounds that modulate ACRP30 polypeptide fragment activity. In some cases it may be important to identify compounds that modulate some but not all of the ACRP30 polypeptide fragment activities, although preferably all activities are modified.

The term "increasing" as used herein refers to the ability of a compound to increase an ACRP30 polypeptide fragment activity in some measurable way compared to the effect of an ACRP30 polypeptide

fragment in its absence. As a result of the presence of the compound leptin binding and/or uptake might increase, for example, as compared to controls in the presence of the ACRP30 polypeptide fragment alone. Preferably, an increase in activity is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% compared to the level of activity in the presence of the ACRP30 fragment.

Similarly, the term "decreasing" as used herein refers to the ability of a compound to decrease an activity in some measurable way compared to the effect of an ACRP30 fragment in its absence. For example, the presence of the compound decreases the plasma concentrations of FFA, TG, and glucose in mice. Also as a result of the presence of a compound leptin binding and/or uptake might decrease, for example, as compared to controls in the presence of the ACRP30 fragment alone. Preferably, a decrease in activity is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% as compared to the level of activity in the presence of the ACRP30 fragment alone.

The invention features a method for identifying a potential compound to modulate body mass in individuals in need of modulating body mass comprising: a) contacting a cell with a gACRP30 fragment and a candidate compound; b) detecting a result selected from the group consisting of LSR modulation, leptin modulation, lipoprotein modulation; FFA oxidation modulation; and c) wherein said result identifies said potential compound if said result differs from said result when said cell is contacted wit h the gACRP30 polypeptide fragment alone.

In preferred embodiments, said contacting further comprises a molecule that binds said LSR. Preferably said binding molecule is selected from the group consisting of cytokine, lipoprotein, free fatty acids, and C1q, and more preferably said cytokine is leptin, and most preferably said leptin is a leptin polypeptide fragment as described in US Provisional application No. 60/155,506 hereby incorporated by reference herein in its entirety including any figures, drawings, or tables.

In further preferred embodiment, said LIGAND is selected from the group of polypeptides comprising Table 2. In other preferred embodiments, said LIGAND is mouse, most preferably ACRP30. In other preferred embodiments, said LIGAND is human, most preferably APM1, C2P, or D2P. In yet further preferred embodiments, said LIGAND is particularly most preferably APM1.

In further preferred embodiment, said LIGAND polypeptide fragments comprise said LIGAND, wherein said LIGAND is selected from the group of polypeptides comprising Table 2. In other preferred embodiments, said LIGAND polypeptide fragment is mouse, most preferably gACRP30 polypeptide fragment. In other preferred embodiments, said LIGAND polypeptide fragment is human, most preferably gAPM1, gC2P, or gD2P polypeptide fragment. In yet further preferred embodiments, said LIGAND polypeptide fragment is particularly most preferably gAPM1. In other preferred embodiments, said cell is selected from the group consisting of PLC, CHO-K1, Hep3B, and HepG2.

In yet other preferred embodiments, said lipoprotein modulation is selected from the group consisting of binding, uptake, and degradation. Preferably, said modulation is an increase in said

binding, uptake, or degradation. Alternatively, said modulation is a decrease in said binding, uptake, or degradation.

In other preferred embodiments, leptin modulation is selected from the group consisting of binding, uptake, degradation, and transport. Preferably, said modulation is an increase in said binding, uptake, degradation, or transport. Alternatively, said modulation is a decrease in said binding, uptake, degradation, or transport. Preferably, said transport is across a blood-brain barrier.

In yet other preferred embodiments, said LSR modulation is expression on the surface of said cell. Preferably, said detecting comprises FACS, more preferably said detecting further comprises antibodies that bind specifically to said LSR, and most preferably said antibodies bind specifically to the carboxy terminus of said LSR.

In still other preferred embodiments, said potential compound is selected from the group consisting of peptides, peptide libraries, non-peptide libraries, peptoids, fatty acids, lipoproteins, medicaments, antibodies, small molecules, and proteases. Other characteristics and advantages of the invention are described in the Examples. These are meant to be exemplary only, and not to limit the invention in any way. Throughout this application, various publications, patents and published patent applications are cited. The disclosures of these publications, patents and published patent specifications referenced in this application are hereby incorporated by reference into the present disclosure.

Assays for Identifying Antagonists of gLIGAND Binding to FRADJ and/or CRYPTIC

The invention features methods of screening for one or more antagonist compounds that block the binding of gLIGAND polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment. Preferred said gLIGAND polypeptide fragment is gAPM1 polypeptide fragment comprised of amino acids 93-244 or 101-244, gC2P polypeptide fragment comprised of amino acids 181-333 or 190-333, or gD2P polypeptide fragment comprised of amino acids 181-333 or 190-333, wherein said numbering of said amino acids within said LIGAND amino acid sequence is understood to be taken from said LIGAND amino acid sequence presented in Table 2. Most preferred gLIGAND polypeptide fragment is gAPM1 polypeptide fragment comprised of amino acids 93-244 or 101-244. Preferred FRADJ and/or CRYPTIC polypeptide fragment is soluble and comprises the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide.

Preferred said compound is a polypeptide.

Other preferred said compound is a polypeptide fragment. Preferred said polypeptide fragment is LIGAND polypeptide fragment. Preferred said LIGAND polypeptide fragment is APM1, C2P, or D2P polypeptide fragment. Further preferred said LIGAND polypeptide fragment is APM1 polypeptide fragment comprised of amino acids 108-142, 153-193, 165-176 or 214-227 or further fragments thereof, C2P polypeptide fragment comprised of amino acids 197-233, 244-284, 256-267 or 304-317 or further fragments thereof, or D2P polypeptide fragment comprised of amino acids 197-233, 244-284, 256-267 or 304-317 or further fragments thereof. Particularly preferred polypeptide fragment is APM1 polypeptide fragment. Further particularly preferred polypeptide fragment is APM1 polypeptide fragment com prised of amino acids 108-142, 153-193, 165-176 or 214-227 or further fragments thereof. It is understood that said numbering of said amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2.

Other preferred said compound is peptide.

Other preferred said compound is protein.

Other preferred said compound is antibody. Preferred said antibody is antibody that specifically binds to FRADJ and/or CRYPTIC polypeptide fragment. More preferred said antibody specifically binds to FRADJ and/or CRYPTIC polypeptide fragment comprising the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide. Further preferred said antibody is antibody that specifica lly binds to LIGAND polypeptide fragment. Particularly preferred said antibody is antibody that specifically binds to APM1, C2P, or D2P polypeptide fragment. Further particularly preferred said antibody is antibody that specficially binds to APM1 polypeptide fragment comprised of amino acids 92-244, 101-244, 108-142 or 167-193, C2P polypeptide fragment comprised of amino acids 181-333, 190-333, 197-233 or 258-284, or D2P polypeptide fragment comprised of amino acids 181-333, 190-333, 197-233 or 258-284. Most particularly preferred said antibody is antibody that specifically binds to APM1 polypeptide fragment. Further most particularly preferred said antibody is antibody that specifically binds to APM1 polypeptide fragment comprised of amino acids 92-244, 101-244, 108-142 or 167-193. It is understood that said numbering of said amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2.

Other preferred said compound is carbohydrate.

Other preferred said compound is lipid.

Other preferred said compound is small molecular weight organic compound.

Other preferred said compound is small molecular weight inorganic compound.

An example of said method of screening for one or more antagonist compound that blocks the binding of gLIGAND polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment comprised of amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4 is enzyme-linked immunosorbent assay (ELISA) comprising: a) incubating and thereby contacting immobilized FRADJ and/or CRYPTIC polypeptide fragment comprised of amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4 with or without a candidate compound; b) further contacting said immobilized FRADJ and/or CRYPTIC polypeptide fragment that has been contacted with said candidate compound with biotinylated-gLIGAND polypeptide fragment; c) contacting biotinylated-gLIGAND polypeptide fragment bound to said immobilized FRADJ and/or CRYPTIC polypeptide fragment with streptavidin-conjugated enzyme; d) contacting bound streptavidin-conjugated enzyme with substrate of said enzyme, wherein action of said enzyme on said substrate results in a color change; and e) detecting the result, wherein said result identifies said compound as an antagonist if the extent of color change is reduced on incubation of said immobilized FRADJ and/or CRYPTIC polypeptide fragment with said compound.

Other example of said method of screening for one or more antagonist compound that blocks the binding of gLIGAND polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment comprised of amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4 is enzyme-linked immunosorbent assay (ELISA) comprising: a) incubating and thereby contacting immobilized gLIGAND polypeptide fragment with or without a candidate compound; b) further contacting said immobilized gLIGAND polypeptide fragment that has been contacted with said candidate compound with biotinylated said FRADJ and/or CRYPTIC polypeptide fragment; c) contacting said biotinylated said FRADJ and/or CRYPTIC polypeptide fragment bound to said immobilized gLIGAND polypeptide fragment with streptavidin-conjugated enzyme; d) contacting bound streptavidin-conjugated enzyme with substrate of said enzyme, wherein action of said enzyme on said substrate results in a color change; and e) detecting the result, wherein said result identifies said compound as an antagonist if the extent of color change is reduced on incubation of said immobilized gLIGAND polypeptide fragment with said compound.

Said examples of screening for one or more antagonist compound that blocks the binding of LIGAND polypeptide or polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment by ELISA are well known to those of ordinary skill in the art. Those of ordinary skill in the art can furthermore devise alternative assays of screening for one or more antagonist compound that blocks the binding of LIGAND polypeptide or polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment.

Assays for Identifying Antagonists of Activity on Binding of gLIGAND Polypeptide Fragment to FRADJ and/or CRYPTIC Polypeptide Fragment

The invention features methods of screening compounds for one or more antagonists of activity on binding of gLIGAND polypeptide fragment to FRADJ and/or CRYPTIC polypeptide fragment, wherein said activity is selected from but not restricted to lipid partitioning, lipid metabolism, and insulin-like activity. Preferred said gLIGAND polypeptide fragment is gAPM1 polypeptide fragment comprised of amino acids 92-244 or 101-244, gC2P polypeptide fragment comprised of amino acids 181-333 or 190-333, or gD2P polypeptide fragment comprised of amino acids 181-333 or 190-333, wherein said numbering of said amino acids within said LIGAND amino acid sequence is understood to be taken from said LIGAND amino acid sequence presented in Table 2. Most preferred LIGAND polypeptide fragment is gAPM1 polypeptide fragment comprised of amino acids 92-244 or 101-244. Preferred said FRADJ and/or CRYPTIC polypeptide fragment is mature FRADJ and/or CRYPTIC polypeptide absent the signal peptide. Further preferred said FRADJ and/or CRYPTIC polypeptide fragment is mature FRADJ and/or CRYPTIC polypeptide absent the signal peptide, wherein said mature FRADJ and/or CRYPTIC polypeptide absent the signal peptide comprises amino acids 28-129 of SEQ ID NO:2. Particularly preferred is said mature FRADJ and/or CRYPTIC polypeptide, wherein said mature FRADJ and/or CRYPTIC polypeptide is expressed as a transmembrane protein at the cell surface.

Preferred said compound is a polypeptide.

Other preferred said compound is a polypeptide fragment. Preferred said polypeptide fragment is LIGAND polypeptide fragment. Preferred said LIGAND polypeptide fragment is APM1, C2P, or D2P polypeptide fragment. Further preferred said LIGAND polypeptide fragment is APM1 polypeptide fragment comprised of amino acids 108-142, 167-193 or 216-228 or further fragments thereof, C2P polypeptide fragment comprised of amino acids 197-233, 258-284 or 306-318 or further fragments thereof, or D2P polypeptide fragment comprised of amino acids 197-233, 258-284 or 306-318 or further fragments thereof. Particularly preferred polypeptide fragment is APM1 polypeptide fragment. Further particularly preferred polypeptide fragment is APM1 polypeptide fragment comprised of amino acids 108-142, 167-193 or 216-228 or further fragments thereof. It is understood that said numbering of said amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2. Also particularly preferred is FRADJ and/or CRYPTIC polypeptide fragment, wherein said FRADJ and/or CRYPTIC polypeptide fragment is soluble and comprises the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide.

Other preferred said compound is peptide.

Other preferred said compound is protein.

Other preferred said compound is antibody. Preferred said antibody is antibody that specifically binds to FRADJ and/or CRYPTIC polypeptide fragment. More preferred said antibody specifically binds to FRADJ and/or CRYPTIC polypeptide fragment comprising the extracellular domain of mature FRADJ and/or CRYPTIC polypeptide. Particularly preferred soluble fragment of FRADJ and/or CRYPTIC comprises amino acids 28-74, 28-76 or 28-77 of SEQ ID NO:2 or 4, where it is understood that amino acid 21 is predicted to be the N-terminal amino acid of the mature FRADJ and/or CRYPTIC polypeptide absent the putative signal peptide. Further preferred said antibody is antibody that specifically binds to LIGAND polypeptide fragment. Particularly preferred said antibody is antibody that specifically binds to APM1, C2P, or D2P polypeptide fragment. Further particularly preferred said antibody is antibody that specficially binds to APM1 polypeptide fragment comprised of amino acids 92-244, 101-244, 108-142 or 153-193, C2P polypeptide fragment comprised of amino acids 181-333, 190-333, 197-233 or 244-284, or D2P polypeptide fragment comprised of amino acids 181-333, 190-333, 197-233 or 244-284. Most particularly preferred said antibody is antibody that specifically binds to APM1 polypeptide fragment. Further most particularly preferred said antibody is antibody that specifically binds to APM1 polypeptide fragment comprised of amino acids 92-244, 101-244, 108-142 or 153-193. It is understood that said numbering of said amino acids within said LIGAND amino acid sequence is taken from said LIGAND amino acid sequence presented in Table 2.

Other preferred said compound is carbohydrate.

Other preferred said compound is lipid.

Other preferred said compound is small molecular weight organic compound.

Other preferred said compound is small molecular weight inorganic compound.

The invention further features methods of screening compounds for said antagonist of said mature FRADJ and/or CRYPTIC polypeptide fragment comprising: a) contacting cells expressing at the cell surface said mature FRADJ and/or CRYPTIC polypeptide fragment or without said compound; b) adding said gLIGAND polypeptide fragment; c) detecting a result on the basis of activity, wherein said activity is selected from but not restricted to lipid partitioning, lipid metabolism, and insulin-like activity; and d) wherein said result identifies said compound as said antagonist if said result with said compound opposes said result without said compound. Exemplary assays that may be used are described in Example 4.

EXAMPLES

The following Examples are provided for illustrative purposes and not as a means of li mitation. One of ordinary skill in the art would be able to design equivalent assays and methods based on the disclosure herein all of which form part of the instant invention.

It should be noted that the term full-length ACRP30 polypeptide used throughout the specification is intended to encompass the protein homologs ACRP30 (Scherer et al (1995) J Biol. Chem. 270:26746-9), ACRP30 (Hu et al (1996) J Biol Chem 271:10697-10703) and the human homolog APM1 (Maeda et al (1996) Biochem Biophys Res Commun 221:289-9) or GBP28 (Nakano et al (1996) J Biochem (Tokyo) 120:803-812). ACRP30 is also intended to encompass other homologs. ACRP30 is also intended to encompass other LIGANDS, wherein said LIGAND is selected from the group of polypeptides comprising Table 2. ACRP30 is preferably intended to encompass APM1.

EXAMPLE 1: Production of Recombinant ACRP30

An exemplary method for generating recombinant ACRP30 is given below. Although the method describes the production of the mouse analog, a person with skill in the art would be able to use the information provided to produce other ACRP30 analogs, including but not limited to the human analog.

The recombinant ACRP30 analog is cloned in pTRC His B (Invitrogen) between BamH1 and Xho1 and maintained in *E. coli* DH5-alpha. The sequence of the ACRP30 insert corresponds to ACRP 30 genbank U37222 bases 88 to 791 except in position 382 where in #3 G replaces A found in ACRP 30 (V instead of M). The corresponding nucleotide in ACRP30 U49915 is G as in clone #3. The ami no acid V is also conserved in the human sequence APM1 D45371.

Culture:

Plate out bacteria in LB agar media containing 100 µg/mL ampicillin. Inoculate 1 colony into 5 mL media (no agar) at 37 °C overnight. Add 2 mL of this initial culture into 500 mL Er lenmeyer flasks containing 200 mL LB media and 100 µg/mL ampicillin. Incubate at 37 °C in an orbital shaker until the OD_{600} = 0.2. Add IPTG to a final concentration of 1 mM (stock solution = 1 M). Incubate at 37 °C overnight.

Lysis:

Pellet the bacteria by centrifugation (Sorvall, 3500 rpm, 15 min, 4°C) in a pre-weighed tube.

At 4°C resuspend the pellet in 3 mL/g of lysis buffer

Add 40 µL/g PMSF 10 mM

Add 80 µL/g of lysozyme 10 mg/mL

Incubate 20 min on ice, shaking intermittently

Add 30 µL/g 10% sodium deoxycholate

Incubate at 37°C until the lysate is viscous

Freeze in liquid Nitrogen and thaw at 37°C three times

Sonicate 2X, 30 sec, 25% cycle, 2.5 power level

Centrifuge 30 min, 15000 rpm, 4°C

Recover the supernatant

Note: The lysate can be stored frozen before or after the sonication step.

Batch Purification:

Pack 1 mL of Probond resin (Invitrogen; 1 mlL= 2 mL suspended gel) into a 5 mL column. Wash with 5 mL PBS.

Apply 5 mL bacterial supernatant to the 1 mL of gel. (If volume is very high, use several small columns.)

Wash with 24 mL phosphate buffer, pH 7.8, followed by a wash with 24 mL phosphate buffer, pH 6.

Elute with imidazole buffer and collect fractions of 1 mL.

Analyze fractions by OD at 280 nm or by SDS-PAGE (12.5%; dilution ½ in 2X sample buffer) under reducing conditions (100°C, 5 min)

Pool the fractions containing protein (usually fraction numbers 2-4 for concentrations of 0.8 – 1 mg/mL and fractions 1, 5 and 6 for concentrations of 0.2 – 0.4 mg/mL).

Dialyze thoroughly against 1 X PBS, 24 mM ammonium bicarbonate or 50 mM Tris, pH 7.4 containing 250 nM NaCl. Concentrate by Speed-Vac if needed.

Analyze protein by the Lowry method.

Aliquot and store at –20°C.

Purification On Liquid Chromatography System

Pack 5 mL of Probond resin into a 5 mL column.

Wash with 4 bed volumes of phosphate buffer pH 7.8, 1 mL/min.

Inject 25 mL lysate (filtered on 0.45 μ or centrifuged at 3000 rpm, 30 min, 4°C, Beckman Allegra 6R) at 0.5 mL/min.

Wash with 4 bed volumes of phosphate buffer, pH 7.8 at 1 mL/min.

Wash with 12 bed volumes of phosphate buffer pH 5.5 at 1 mL/min.

Elute bound fraction with phosphate buffer, pH 5.5, containing 1 M imidazole at 1 mL/min.

Collect fractions, dialyze and analyze protein as described for batch purification, steps 7-9.

EXAMPLE 2: Generation of Globular ACRP30 by Enzymatic Cleavage

Incubate purified ACRP30 (obtained as described above or through equivalent method) with acetylated Trypsin-Type V-S from Bovine Pancreas (Sigma E.C. = 3.4.21.4) at 400 u/mg protein at 25 °C for 10 min.

Stop reaction by running the sample over a Poly-Prep Column (Biorad 731-1550) at + 4°C containing immobilized Trypsin inhibitor.

Collect 1.0 mL fractions. Determine protein concentration.

Pool the protein containing fractions and dialyze extensively against PBS using dialysis tubing with M.W. cutoff = 10,000 da.

Concentrate on Amicon YM-10 Centricon Filter (Millipore, M.W. cutoff = 10,000 da). Sterile filter.

Determine final protein concentration using Markwell's modified Lowry procedure (1981) or BCA protein assay (Pierce Chemical Co, Rockford, IL) and BSA as standard.

Check purity and efficiency of cleavage by SDS – PAGE analysis using a 4-20% gradient gel. The intact ACRP30 migrates as a single band at approximately 37 kDa apparently due to co-transcribed vector sequences attached to the histidine tag at the N-terminus of ACRP30, and forms a dimer at 74 kDa. The cleaved ACRP30 forms a band at approx. 18 kDa (gACRP30). Additional degradation products, all smaller than 10 kDa are also generated from the N-terminal region. These are separated from the desired 18 kDa band by dialysis with semipermeable membranes with a MW cutoff of 10,000. There are two potential cleavage sites for gACRP30. The actual cleavage site has been identified as the

one after amino acid 103 (amino acid 100 for human gACRP30 or APM1). That is, the N-terminus of the gACRP30 cleavage product is Lys 104 (Lys 101 for human gACRP30 or APM1).

Other enzymatic/proteolytic methods can also be used that yield similar products, *e.g.* clostripain. Other preferred enzymes would preferably cleave ACRP30 at a site close to the junction between the collagen-like tail and the globular head (about amino acid 108 for human gACRP30 and about amino acid 111 for murine gACRP30), preferably permit the reaction to be easily stopped, preferably be easily removed using an immobilized inhibitor, or similar method, and preferably cuts the N-terminal fragment into small pieces (less than 10,000 MW). The cleavage preferably results in the presence of no more than 6 collagen repeats, more preferably 3 collagen repeats, and most preferably no collagen repeats. A collagen repeat consists of GLY-X-Y. A determination of whether an active gACRP30 has been generated can be checked using the *in vitro* and *in vivo* assays described herein (Examples 4-6, 8-10).

EXAMPLE 3: Generation of gACRP30 by Recombinant DNA Methodology

Restriction Site Cloning

A first approach is to look for unique restriction sites near the beginning of the globular head region (nucleic acid sequences of mouse and human ACRP30 polypeptides are provided in the sequence listing). If present, it can be used to cleave the 5' collagen-like region from the globular head region. If a unique site is not present, it is also possible, although more difficult, to do this using restriction enzymes that cut in more than one location by doing partial digestions. The 3' end of the globular head can be cut from its vector backbone using an appropriate enzyme. The globular head can then be cloned into an expression vector and constructs containing the correct fragments can be identified. For ACRP30, Tau I seems to be a unique enzyme that would separate the collagen tail from the globular head.

PCR Cloning

Another approach is to PCR the region of interest from the intact sequence (if cDNA is available) using primers with restriction sites on the end so that PCR products can be directly cloned into vectors of interest. Alternatively, gACRP30 can also be generated using RT-PCR to isolate it from adipose tissue RNA.

E. coli Vector

For example, the ACRP30 globular region can be cloned into pTrcHisB, by putting a Bam HI site on the sense oligo and a Xho I site on the antisense oligo. This allows isolation of the PCR product, digestion of that product, and ligation into the pTrcHisB vector that has also been digested with Bam HI

and Xho I. The vector, pTrcHisB, has an N-terminal 6-Histidine tag, that allows purification of the over expressed protein from the lysate using a Nickel resin column. The pTrcHisB vector is used for over-expression of proteins in *E. coli*.

Exemplary oligos for cloning into the *E. coli* vector include:

A) ACRP30 sense CTTAGTGGATCCCGCTTATGTGTATCGCTCAG 6 base pairs from the left there is a 6 bp BamHI site. Thus the region that is homologous to the gene begins at nucleotide 13.

B) ACRP30 antisense GCTGTTCTCGAGTCAGTTGGTATCATGG 6 base pairs from the left there is a 6 bp. XhoI site. Thus the region that is homologous to the gene begins at nucleotide 13.

The following are exemplary PCR conditions.

Final concentrations in the reaction are:

1X PE Biosystems buffer A

1.5mM $MgCl_2$

200uM of each dNTP (dATP, dCTP, dGTP, dTTP)

2.5 Units of Amplitaq Gold from PE Biosystems

0.4uM of each primer (sense and antisense)

10 ng of plasmid template

Cycling parameters:

95°C 10min --- 1 cycle

95°C 30sec

56°C 30sec

72°C 30sec

repeat above 3 steps for 30 cycles

72°C 7min --- 1 cycle.

BAC Vector

The globular head can also be over expressed in a Baculovirus system using the 6xHis Baculovirus kit (Pharmingen), for example. The ACRP30 globular region is cloned into the appropriate

vector using enzymes available in the multiple cloning site. This allows over-expression of the protein in a eukaryotic system that has some advantages over the *E.coli* system, including: Multiple gene expression, Signal peptide cleavage, Intron splicing, Nuclear transport, Functional protein, Phosphorylation, Glycosylation, and Acylation.

Exemplary oligos for cloning into the Baculovirus vector are the following:

a) ACRP30 sense CTTAGTGAATTCGCTTATGTGTATCGCTCAGA 6 base pairs from the left there is a 6 bp. EcoRI site. Thus the region that is homologous to the gene begins at nucleotide 13.

b) ACRP30 antisense GCTGTTCTGCAGTCAGTTGGTATCATGG 6 base pairs from the left there is a 6 bp. PstI site. Thus the region that is homologous to the gene begins at nucleotide 13.

The following are exemplary PCR conditions.

Final concentrations in the reaction are:

1X PE Biosystems buffer A

1.5mM $MgCl_2$

200uM of each dNTP (dATP, dCTP, dGTP, dTTP)

2.5 Units of Amplitaq Gold from PE Biosystems

0.4uM of each primer (sense and antisense)

10 ng of plasmid template

Cycling parameters:

95°C 10min --- 1 cycle

95°C 30sec

60°C 30sec

72°C 30sec

repeat above 3 steps for 30 cycles

72°C 7min --- 1 cycle.

Mammalian Vector

Globular ACRP30 can also be cloned into a mammalian expression vector and expressed in and purified from mammalian cells, for example 3T3-L1 cells (undifferentiated adipocyte precursors). The globular head is then generated in an environment very close to its endogenous environment. However, this is not necessarily the most efficient way to make protein.

EXAMPLE 4: In Vitro Tests of Obesity-related Activity

The activity of various preparations and various sequence variants of gACRP30 polypeptide fragments are assessed using various *in vitro* assays including those provided below. These assays are also exemplary of those that can be used to develop gACRP30 polypeptide fragment ANTAGONISTS and AGONISTS. To do that, the effect of gACRP30 polypeptide fragments in the above assays, e.g. on leptin and/or LSR activity, in the presence of the candidate molecules would be compared with the effect of gACRP30 polypeptide fragments in the assays in the absence of the candidate molecules. Since gACRP30 polypeptide fragments have been shown to reduce body weight in mice on a high-cafeteria diet (Example 5), these assays also serve to identify candidate treatments for reducing (or increasing) body weight.

Liver Cell Line:

Tests of efficacy of gACRP30 polypeptide fragments on LSR can be performed using liver cell lines, including for example, PLC, HepG2, Hep3B (human), Hepa 1-6, BPRCL (mouse), or MCA-RH777, MCA-RH8994 (rat). For human cell lines, APM1 and globular APM1 would be used preferentially; for rodents, full-length and globular ACRP30 would be used preferentially.

BPRCL mouse liver cells (ATCC Repository) were plated at a density of 300,000 cells/well in 6-well plates (day 0) in DMEM (high glucose) containing glutamine and penicillin-streptomycin (Bihain & Yen, 1992). Media was changed on day 2. On day 3, the confluent monolayers were washed once with phosphate-buffered saline (PBS, pH 7.4) (2 mL/well). Cells were incubated at 37°C for 30 min with increasing concentrations of recombinant ACRP30 or globular ACRP30 (gACRP30) in DMEM containing 0.2% (w/v) BSA, 5 mM Hepes, 2 mM $CaCl_2$, 3.7 g/L sodium bicarbonate, pH 7.5. Incubations were continued for 3 h at 37°C after addition of 10 ng/mL ^{125}I-mouse leptin (specific activity, 22100 cpm/ng). Monolayers were washed 2 times consecutively with PBS containing 0.2% BSA, followed by 1 wash with PBS/BSA, and then 2 times consecutively with PBS. Cells were lysed with 0.1 N NaOH containing 0.24 mM EDTA. Lysates were collected into tubes, and counted in a gamma-counter.

The results indicate that gACRP30 polypeptide fragments are at least 30% more efficient than ACRP30 in increasing leptin uptake in a liver cell line. This assay could be used to determine the efficiency of gACRP30 polypeptide fragments and related compounds (or AGONISTS or ANTAGONISTS) to increase or decrease leptin uptake into the liver, as well as the mechanism by which the gACRP30 polypeptide fragment/compound exerts this effect.

Blood Brain Barrier Model:

The effect of gACRP30 polypeptide fragments on leptin transport in the brain can be determined using brain-derived cells. One method that is envisioned is to use the blood/brain barrier model described by Dehouck, *et al* (J Neurochem 54:1798-801, 1990; hereby incorporated herein by reference in its entirety including any figures, tables, or drawings) that uses a co-culture of brain capillary endothelial cells and astrocytes to test the effects of gACRP30 polypeptide fragments on leptin (or other molecules) transport via LSR or other receptors.

This assay would be an indicator of the potential effect of gACRP30 polypeptide fragments on leptin transport to the brain and could be used to screen gACRP30 polypeptide fragment variants for their ability to modulate leptin transport through LSR or other receptors in the brain. In addition, putative AGONISTS and ANTAGONISTS of the effect of gACRP30 polypeptide fragments on leptin transport through LSR or other receptors could also be screened using this assay. Increased transport of leptin across the blood/brain barrier would presumably increase its action as a satiety factor.

FACS Analysis of LSR Expression

The effect of gACRP30 polypeptide fragments on LSR can also be determined by measuring the level of LSR expression at the cell surface by flow surface cytometry, using anti-LSR antibodies and fluorescent secondary antibodies. Flow cytometry is a laser-based technology that is used to measure characteristics of biological particles. The underlying principle of flow cytometry is that light is scattered and fluorescence is emitted as light from the excitation source strikes the moving particles.

This is a high through-put assay that could be easily adapted to screen ACRP30 and gACRP30 polypeptide fragments and variants as well as putative AGONISTS or ANTAGONISTS of gACRP30 polypeptide fragments. Two assays are provided below. The antibody, cell-line and gACRP30 polypeptide fragment analog would vary depending on the experiment, but a human cell-line, human anti-LSR antibody and globular APM1 could be used to screen for variants, AGONISTS, and ANTAGONISTS to be used to treat humans.

Assay 1:

Cells are pretreated with either intact ACRP30 or gACRP30 polypeptide fragments (or untreated) before harvesting and analysis by FACS. Cells are harvested using non-enzymatic dissociation solution (Sigma), and then are incubated for 1 h at 4°C with a 1:200 dilution of anti-LSR 81B or an irrelevant anti-serum in PBS containing 1% (w/v) BSA. After washing twice with the same buffer, goat anti-rabbit FITC-conjugated antibody (Rockland, Gilbertsville, PA) is added to the cells, followed by a further incubation for 30 min at 4 °C. After washing, the cells are fixed in 2% formalin. Flow cytometry analysis is done on a FACSCalibur cytometer (Becton-Dickinson, Franklin Lakes, NJ).

The *in vitro* Liver Cell Line assay (described above) has shown that LSR activity (leptin binding) increases with increasing concentrations of gACRP30 polypeptide fragments. While not wishing to be bound by any particular theory, this could either be the result of an increased number of LSR binding sites on the cell surface, or a change in affinity for leptin. The FACS assay would presumably be detecting changes in the number of LSR binding sites, although changes in conformation reflecting changes in affinity might also be detected. Preferably the antibody would be to the C-terminus of LSR.

Assay 2:

Cells are cultured in T175 flasks according to manufacturer's instructions for 48 hours prior to analysis.

Cells are washed once with FACS buffer (1x PBS/2% FBS, filter sterilized), and manually scraped from the flask in 10 mLs of FACS buffer. The cell suspension is transferred to a 15 mL conical tube and centrifuged at 1200 rpm, 4 °C for 5 minutes. Supernatant is discarded and cells are resuspended in 10 mL FACS buffer chilled to 4 °C. A cell count is performed and the cell density adjusted with FACS buffer to a concentration of 1 x 10^6 cells/ mL. One milliliter of cell suspension was added to each well of a 48 well plate for analysis. Cells are centrifuged at 1200 rpm for 5 minutes at 4 °C. Plates are checked to ensure that cells are pelleted, the supernatant is removed and cells resuspended by running plate over a vortex mixer. One milliliter of FACS buffer is added to each well, followed by centrifugation at 1200 rpm for 5 minutes at 4 °C. This described cell washing was performed a total of 3 times.

Primary antibody, titered in screening experiments to determine proper working dilutions (for example 1:25, 1:50, 1:100, 1:200, 1:400, 1:500, 1:800, 1:1000, 1:2000, 1:4000, 1:5000, or 1:10000), is added to cells in a total volume of 50 µL FACS buffer. Plates are incubated for 1h at 4°C protected from light. Following incubation, cells are washed 3 times as directed above. Appropriate secondary antibody, titered in screening experiments to determine proper working dilutions (for example 1:25, 1:50, 1:100, 1:200, 1:400, 1:500, 1:800, 1:1000, 1:2000, 1:4000, 1:5000, or 1:10000), is added to cells in a total volume of 50 µL FACS buffer. Plates are incubated for 1h at 4°C protected from light. Following incubation, cells are washed 3 times as directed above. Upon final wash, cells are resuspended in 500 µL

FACS buffer and transfered to a FACS acquisition tube. Samples are placed on ice protected from light and analyzde within 1 hour.

Cellular Binding and Uptake of gACRP30 as Detected by Fluorescence Microscopy

Fluorecein isothiocyanate (FITC) conjugation of gACRP30: Purified gACRP30 at 1 mg/mL concentration was labeled with FITC using Sigma's FluoroTag FITC conjugation kit (Stock No. FITC-1). Protocol outlined in the Sigma Handbook for small scale conjugation was followed for gACRP30 labeling.

Cell Culture: C2C12 mouse skeletal muscle cells (ATCC, Manassas, VA CRL-1772) and Hepa-1-6 mouse hepatocytes (ATCC, Manassas, VA CRL-1830) were seeded into 6 well plates at a cell density of 2x105 cells per well. C2C12 and Hepa-1-6 cells were cultured according to repository's instructions for 24-48 hours prior to analysis. Assay was performed when cells were 80% confluent.

FITC labeled gACRP30 cellular binding and uptake using microscopy: C2C12 and Hepa 1-6 cells were incubated in the presence/absence of antibody directed against human LSR (81B: N-terminal sequence of human LSR; does not cross react with mouse LSR and 93A: c-terminal sequence, cross reacts with mouse LSR) or an antiserum directed against gC1qr (953) for 1 hour at 37°C, 5% CO2. LSR antibodies were added to the media at a concentration of 2 µg/mL. The anti-gC1qr antiserum was added to the media at a volume of 2.5 µL undiluted serum (high concentration) or 1:100 dilution (low concentration). Following incubation with specified antibody, FITC-gACRP30 (50 nM/mL) was added to each cell culture well. Cells were again incubated for 1 hour at 37°C, 5% CO2. Cells were washed 2x with PBS, cells were scraped from well into 1 mL of PBS. Cell suspension was transferred to an eppendorf tube and centrifuged at 1000 rpm for 2 minutes. Supernatant was removed and cells resuspended in 200 µL of PBS. Binding and uptake of FITC-gACRP30 was analyzed by fluorescence microscopy under 40X magnification.

Analysis of C2C12 and Hepa 1-6 cells reveals identical phenotypes with respect to FITC-gACRP30 binding and uptake profiles both in the presence or absence of LSR antibodies. FITC-gACRP30 appears to be localized within vesicles in the cytoplasm of both mouse hepatocytes and mouse myoblasts, suggesting that binding and uptake of FITC-gACRP30 is occurring. FITC-gACRP30 uptake appears to be blocked when cells were pre-treated with the anti-LSR antibody that recognizes mouse LSR. However, binding of FITC- gACRP30 to the cell surface does occur in a small portion of the cells (C2C12 and Hepa 1-6). At low concentration of the gC1qr antiserum, FITC-gACRP30 appears to be localized within vesicles in the cytoplasm of both cell types, similarly to the phenotype of cells that have not received antibody pre-treatment prior to addition of FITC-gACRP30. FITC-gACRP30 uptake and binding phenotype is not affected by pre-treatment with an LSR antibody that does not recognize mouse

LSR. Together, these data suggest that uptake of FITC-gACRP30 can be blocked by a human LSR antibody which cross-reacts with mouse LSR. However, this phenotype is not reproduced with other non cross-reactive LSR antibodies. Thus, this assay may be useful for identifying agents that facilitate or prevent the uptake and/or binding of ACRP30 or gACRP30 polypeptide fragments to cells.

Effect on LSR as a Lipoprotein Receptor

The effect of gACRP30 on the lipoprotein binding, internalizing and degrading activity of LSR can also be tested. Measurement of LSR as lipoprotein receptor is described in Bihain & Yen, ((1992) Biochemistry May 19;31(19):4628-36; hereby incorporated herein in its entirety including any drawings, tables, or figures). The effect of gACRP30 on the lipoprotein binding, internalizing and degrading activity of LSR (or other receptors) can be compared with that of intact ACRP30, with untreated cells as an additional control. This assay can also be used to screen for active and inhibitory variants of gACRP30, as well as AGONISTS and ANTAGONISTS of obesity-related activity.

Human liver PLC cells (ATCC Repository) were plated at a density of 300,000 cells/well in 6-well plates (day 0) in DMEM (high glucose) containing glutamine and penicillin-streptomycin (Bihain & Yen, 1992). Media was changed on day 2. On day 3, the confluent monolayers were washed once with phosphate-buffered saline (PBS, pH 7.4) (2 mL/well). Cells were incubated at 37°C for 30 min with 10 ng/mL human recombinant leptin in DMEM containing 0.2% (w/v) BSA, 5 mM Hepes, 2 mM $CaCl_2$, 3.7 g/L sodium bicarbonate, pH 7.5, followed by another 30 min incubation at 37°C with increasing concentrations of gACRP30. Incubations were continued for 2 h at 37°C after addition of 0.8 mM oleate and 20 µg/mL ^{125}I-LDL. Monolayers were washed 2 times consecutively with PBS containing 0.2% BSA, followed by 1 wash with PBS/BSA, and then 2 times consecutively with PBS. The amounts of oleate-induced binding, uptake and degradation of ^{125}I-LDL were measured as previously described (Bihain & Yen, 1992, supra). Results are shown as the mean of triplicate determinations.

As shown in Figure 6, the addition of gACRP30 leads to an increased activity of LSR as a lipoprotein receptor. The oleate-induced binding and uptake of LDL appears more affected by gACRP30 as compared to the degradation. This increased LSR activity would potentially result in an enhanced clearance of triglyceride-rich lipoproteins during the postprandial state. Thus, more dietary fat would be removed through the liver, rather than being deposited in the adipose tissue.

This assay could be used to determine the efficiency of a compound (or AGONISTS or ANTAGONISTS) to increase or decrease LSR activity (or lipoprotein uptake, binding and degradation through other receptors), and thus affect the rate of clearance of triglyceride-rich lipoproteins.

Effect on Muscle Differentiation

C2C12 cells (murine skeletal muscle cell line; ATCC CRL 1772, Rockville, MD) are seeded sparsely (about 15-20%) in complete DMEM (w/glutamine, pen/strep, etc) + 10% FCS. Two days later they become 80-90% confluent. At this time, the media is changed to DMEM+2% horse serum to allow differentiation. The media is changed daily. Abundant myotube formation occurs after 3 -4 days of being in 2% horse serum, although the exact time course of C2C12 differentiation depends on how long they have been passaged and how they have been maintained, among other things.

To test the effect of the presence of gACRP30 on muscle differentiation, gACRP30 (1 to 2.5 μg/mL) was added the day after seeding when the cells were still in DMEM w/ 10% FCS. Two days after plating the cells (one day after gACRP30 was first added), at about 80-90% confluency, the media was changed to DMEM+2% horse serum plus gACRP30.

The results show that the addition of gACRP30 causes the cells to begin organizing within one day after its addition. In contrast to the random orientation of the cells not treated with gACRP30, those treated with gACRP30 aligned themselves in relation to each other. In addition, differentiation occurred after only 2 days of gACRP30 treatment, in contrast to the 3 to 4 days needed in its absence.

Effect on Muscle Cell Fatty Acid Oxidation

C2C12 cells were differentiated in the presence or absence of 2 μg/mL gACRP30 for 4 days. On day 4, oleate oxidation rates were determined by measuring conversion of 1-^{14}C-oleate (0.2 mM) to $^{14}CO_2$ for 90 min. C2C12 cells differentiated in the presence of gACRP30 undergo 40% more oleate oxidation than controls differentiated in the absence of gACRP30. This experiment can be used to screen for active fragments and peptides as well as AGONISTS and ANTAGONISTS or activators and inhibitors of ACRP30 and gACRP30 polypeptides.

The effect of gACRP30 on the rate of oleate oxidation was compared in differentiated C2C12 cells (murine skeletal muscle cells; ATCC, Manassas, VA CRL-1772) and in a hepatocyte cell line (Hepa1-6; ATCC, Manassas, VA CRL-1830). Cultured cells were maintained according to manufacturer's instructions. The oleate oxidation assay was performed as previously described (Muoio et al (1999) Biochem J 338:783-791). Briefly, nearly confluent myocytes were kept in low serum differentiation media (DMEM, 2.5% Horse serum) for 4 days, at which time formation of myotubes became maximal. Hepatocytes were kept in the same DMEM medium supplemented with 10% FCS for 2 days. One hour prior to the experiment the media was removed and 1 mL of preincubation media (MEM, 2.5% Horse serum, 3 mM glucose, 4 mM Glutamine, 25 mM Hepes, 1% FFA free BSA, 0.25 mM Oleate, 5 μg/mL gentamycin) was added. At the start of the oxidation experiment ^{14}C-Oleic acid (1μCi/mL, American Radiolabeled Chemical Inc., St. Louis, MO) was added and cells were

incubated for 90 min at 37°C in the absence/presence of 2.5 µg/mL gACRP30. After the incubation period 0.75 mL of the media was removed and assayed for ^{14}C-oxidation products as described below for the muscle FFA oxidation experiment.

Oleate oxidation in C2C12 cells determined over 90 min increased significantly (39%; p = 0.036, two-tailed t-Test) in cells treated with gACRP30. In contrast, no detectable increase in the rate of FFA oxidation was seen in hepatocytes incubated with gACRP30.

Triglyceride and Protein Analysis following Oleate Oxidation in cultured cells

Following transfer of media for oleate oxidation assay, cells were placed on ice. To determine triglyceride and protein content, cells were washed with 1 mL of 1x PBS to remove residual media. To each well 300 µL of cell dissociation solution (Sigma) was added and incubated at 37°C for 10 min. Plates were tapped to loosen cells, and 0.5 mL of 1x PBS was added. The cell suspension was transferred to an eppendorf tube, each well was rinsed with an additional 0.5 mL of 1x PBS, and was transferred to appropriate eppendorf tube. Samples were centrifuged at 1000 rpm for 10 minutes at room temperature. Supernatant was discarded and 750 µL of 1x PBS/2% chaps was added to cell pellet. Cell suspension was vortexed and place on ice for 1 hour. Samples were then centrifuged at 13000 rpm for 20 min at 4°C. Supernatants were transferred to new tube and frozen at –20°C until analyzed. Quantitative measure of triglyceride level in each sample was determined using Sigma Diagnostics GPO-TRINDER enzymatic kit. The procedure outlined in the manual was adhered to, with the following exceptions: assay was performed in 48 well plate, 350 µL of sample volume was assayed, control blank consisted of 350 µL PBS/2% chaps, and standard contained 10 µL standard provide in kit plus 690 µL PBS/2% chaps. Analysis of samples was carried out on a Packard Spectra Count at a wavelength of 550 nm. Protein analysis was carried out on 25 µL of each supernatant sample using the BCA protein assay (Pierce) following manufacturer's instructions. Analysis of samples was carried out on a Packard Spectra Count at a wavelength of 550 nm.

Triglyceride production in both C2C12 and Hepa 1-6 cells did not change significantly in the absence/presence of ACRP30 and gACRP30. The protein content of all cells analyzed was equivalent in the absence/presence of ACRP30 and gACRP30.

EXAMPLE 4: In vitro glucose uptake by muscle cells.

L6 Muscle cells are obtained from the European Culture Collection (Porton Down) and are used at passages 7-11. Cells are maintained in standard tissue culture medium DMEM, and glucose uptake is assessed using [.sup.3 H]-2-deoxyglucose (2DG) with or without gACRP30 in the presence or absence of insulin (10.sup.-8 M) as has been previously described (Walker, P.S. et al. (1990) Glucose transport

activity in L6 muscle cells is regulated by the coordinate control of subcellular glucose transporter distribution, biosynthesis, and mRNA transcription. JBC 265(3):1516-1523; and Kilp, A. et al. (1992) Stimulation of hexose transport by metformin in L6 muscle cells in culture. Endocrinology 130(5):2535-2544, which disclosures are hereby incorporated by reference in their entireties). Uptake of 2DG is expressed as the percentage change compared with control (no added insulin or gACRP30). Values are presented as mean .+-.SEM of sets of 4 wells per experiment. Differences between sets of wells are evaluated by Student's t test, probability values $p<0.05$ are considered to be significant.

EXAMPLE 5: Effect of gACRP30 on Mice Fed a High-Fat Diet

Experiments are performed using approximately 6 week old C57Bl/6 mice (8 per group). All mice are housed individually. The mice are maintained on a high fat diet throughout each experiment. The high fat diet (cafeteria diet; D12331 from Research Diets, Inc.) has the following composition: protein kcal% 16, sucrose kcal% 26, and fat kcal% 58. The fat was primarily composed of coconut oil, hydrogenated.

After the mice are fed a high fat diet for 6 days, micro-osmotic pumps are inserted using isoflurane anesthesia, and are used to provide gACRP30, ACRP30, saline, and an irrelevant peptide to the mice subcutaneously (s.c.) for 18 days. gACRP30 is provided at doses of 50, 25, and 2.5 μg/day; ACRP30 is provided at 100, 50, and 5 μg/day; and the irrelevant peptide is provided at 10 μg/day. Body weight is measured on the first, third and fifth day of the high fat diet, and then daily after the start of treatment. Final blood samples are taken by cardiac puncture and are used to determine triglyceride (TG), total cholesterol (TC), glucose, leptin, and insulin levels. The amount of food consumed per day is also determined for each group.

In a preliminary experiment, mice treated with 2.5 μg/day gACRP30 had significantly lowered body weight.

EXAMPLE 6: Tests of Obesity-related Activity in Humans

Tests of the efficacy of gACRP30 in humans are performed in accordance with a physician's recommendations and with established guidelines. The parameters tested in mice are also tested in humans (*e.g.* food intake, weight, TG, TC, glucose, insulin, leptin, FFA). It is expected that the physiological factors would show changes over the short term. Changes in weight gain might require a longer period of time. In addition, the diet would need to be carefully monitored. Globular ACRP30 would be given in daily doses of about 6 mg protein per 70 kg person or about 10 mg per day. Other doses would also be tested, for instance 1 mg or 5 mg per day up to 20 mg, 50 mg, or 100 mg per day.

EXAMPLE 7: In Vivo Tests for Metabolic-related Activity in Rodent Diabetes Models

As metabolic profiles differ among various animal models of obesity and diabetes, analysis of multiple models is undertaken to separate the effects gACRP30 on hyperglycemia, hyperinsulinemia, hyperlipidemia and obesity. Mutation in colonies of laboratory animals and different sensitivities to dietary regimens have made the development of animal models with non-insulin dependent diabetes associated with obesity and insulin resistance possible. Genetic models such as db/db and ob/ob (See Diabetes, (1982) 31(1): 1-6) in mice and fa/fa in zucker rats have been developed by the various laboratories for understanding the pathophysiology of disease and testing the efficacy of new antidiabetic compounds (Diabetes, (1983) 32: 830-838; Annu. Rep. Sankyo Res. Lab. (1994). 46: 1-57). The homozygous animals, C57 BL/KsJ-db/db mice developed by Jackson Laboratory, US, are obese, hyperglycemic, hyperinsulinemic and insulin resistant (J. Clin. Invest., (1990) 85: 962-967), whereas heterozygous are lean and normoglycemic. In db/db model, mouse progressively develops insulinopenia with age, a feature commonly observed in late stages of human type II diabetes when blood sugar levels are insufficiently controlled. The state of pancreas and its course vary according to the models. Since this model resembles that of type II diabetes mellitus, the compounds of the present invention are tested for blood sugar and triglycerides lowering activities. Zucker (fa/fa) rats are severely obese, hyperinsulinemic, and insulin resistant (Coleman, Diabetes 31:1, 1982; E. Shafrir, in Diabetes Mellitus; H. Rifkin and D. Porte, Jr. Eds. (Elsevier Science Publishing Co., Inc., New York, ed. 4, 1990), pp. 299 - 340), and the fa/fa mutation may be the rat equivalent of the murine db mutation (Friedman et al., Cell 69:217-220, 1992; Truett et al., Proc. Natl. Acad. Sci. USA 88:7806, 1991). Tubby (tub/tub) mice are characterized by obesity, moderate insulin resistance and hyperinsulinemia without significant hyperglycemia (Coleman et al., J. Heredity 81:424, 1990).

Previously, leptin was reported to reverse insulin resistance and diabetes mellitus in mice with congenital lipodystrophy (Shimomura et al. Nature 401: 73-76 (1999). Leptin is found to be less effective in a different lipodystrophic mouse model of lipoatrophic diabetes (Gavrilova et al Nature 403: 850 (2000); hereby incorporated herein in its entirety including any drawings, figures, or tables).

The streptozotocin (STZ) model for chemically-induced diabetes is tested to examine the effects of hyperglycemia in the absence of obesity. STZ-treated animals are deficient in insulin and severely hyperglycemic (Coleman, Diabetes 31:1, 1982; E. Shafrir, in Diabetes Mellitus; H. Rifkin and D. Porte, Jr. Eds. (Elsevier Science Publishing Co., Inc., New York, ed. 4, 1990), pp. 299-340). The monosodium glutamate (MSG) model for chemically-induced obesity (Olney, Science 164:719, 1969; Cameron et al., Cli. Exp. Pharmacol. Physiol. 5:41, 1978), in which obesity is less severe than in the genetic models and develops without hyperphagia, hyperinsulinemia and insulin resistance, is also examined. Finally, a non-chemical, non-genetic model for induction of obesity includes feeding rodents a high fat/high carbohydrate (cafeteria diet) diet ad libitum.

The instant invention encompasses the use of gACRP30 for reducing the insulin resistance and hyperglycemia in any or all of the above rodent diabetes models or in humans with Type I or Type II diabetes or other prefered metabolic diseases described previously or models based on other mammals. In the compositions of the present invention gACRP30 may, if desired, be associated with other compatible pharmacologically-active antidiabetic agents such as insulin, leptin (US provisional application No 60/155,506), or troglitazone, either alone or in combination. Assays include that described previously in Gavrilova et al. ((2000) Diabetes Nov; 49(11):1910-6; (2000) Nature Feb 24;403(6772):850) using A-ZIP/F-1 mice, except that gACRP30 is administered intraperotineally, subcutaneously, intramuscularly or intravenously. The glucose and insulin levels of the mice would be tested, and the food intake and liver weight monitored, as well as other factors, such as leptin, FFA, and TG levels, typically measured in our experiments.

In Vivo Assay for Anti-hyperglycemic Activity of gACRP30

Genetically altered obese diabetic mice (db/db) (male, 7-9 weeks old) are housed (7-9 mice/cage) under standard laboratory conditions at 22 °C and 50% relative humidity, and maintained on a diet of Purina rodent chow and water ad libitum. Prior to treatment, blood is collected from the tail vein of each animal and blood glucose concentrations are determined using One Touch BasicGlucose Monitor System (Lifescan). Mice that have plasma glucose levels between 250 to 500 mg/dl are used. Each treatment group consists of seven mice that are distributed so that the mean glucose levels are equivalent in each group at the start of the study. db/db mice are dosed by micro-osmotic pumps, inserted using isoflurane anesthesia, to provide gACRP30, saline, and an irrelevant peptide to the mice subcutaneously (s.c.). Blood is sampled from the tail vein hourly for 4 hours and at 24, 30 h post-dosing and analyzed for blood glucose concentrations. Food is withdrawn from 0-4 h post dosing and reintroduced thereafter. Individual body weights and mean food consumption (each cage) are also measured after 24 h. Significant differences between groups (comparing gACRP30 treated to saline-treated) are evaluated using Student t-test.

In Vivo Insulin Sensitivity Assay

In vivo insulin sensitivity is examined by utilizing two-step hyperinsulinemic-euglycemic clamps according to the following protocol. Rodents from any or all of the various models described herein are housed for at least a week prior to experimental procedures. Surgeries for the placement of jugular vein and carotid artery catheters are performed under sterile conditions using ketamine and xylazine (i.m.) anesthesia. After surgery, all rodents are allowed to regain consciousness and placed in individual cages. gACRP30 or vehicle is administered through the jugular vein after complete recovery and for the following two days. Sixteen hours after the last treatment, hyperinsulinemic-euglycemic clamps are

performed. Rodents are placed in restrainers and a bolus of 4 μCi [3-^{3}H] glucose (NEN) is administered, followed by a continuous infusion of the tracer at a dose of 0.2 μCi/min (20 μl/min). Two hours after the start of the tracer infusion, 3 blood samples (0.3 ml each) are collected at 10 minute intervals (-20-0 min) for basal measurements. An insulin infusion is then started (5 mU/kg/min), and 100 .mu.l blood samples are taken every 10 min. to monitor plasma glucose. A 30% glucose solution is infused using a second pump based on the plasma glucose levels in order to reach and maintain euglycemia. Once a steady state is established at 5 mU/kg/min insulin (stable glucose infusion rate and plasma glucose), 3 additional blood samples (0.3 ml each) are obtained for measurements of glucose, [3-^{3}H] glucose and insulin (100-120 min.). A higher dose of insulin (25 mU/kg/min.) is then administered and glucose infusion rates are adjusted for the second euglycemic clamp and blood samples are taken at 220-240 min. Glucose specific activity is determined in deproteinized plasma and the calculations of Rd and hepatic glucose output (HGO) are made, as described (Lang et al., Endocrinology 130:43, 1992). Plasma insulin levels at basal period and after 5 and 25 mU/kg/min. infusions are then determined and compared between gACRP30 treated and vehicle treated rodents.

Insulin regulation of glucose homeostasis has two major components: stimulation of peripheral glucose uptake and suppression of hepatic glucose output. Using tracer studies in the glucose clamps, it is possible to determine which portion of the insulin response is affected by gACRP30.

EXAMPLE 8: Tests of Obesity-related Activity in a Murine Lipoatrophic Diabetes Model

Previously, leptin was reported to reverse insulin resistance and diabetes mellitus in mice with congenital lipodystrophy (Shimomura et al. Nature 401: 73-76 (1999); hereby incorporated herein in its entirety including any drawings, figures, or tables). Leptin was found to be less effective in a different lipodystrophic mouse model of lipoatrophic diabetes (Gavrilova et al Nature 403: 850 (2000); hereby incorporated herein in its entirety including any drawings, figures, or tables). The instant invention encompasses the use of ACRP30 or gACRP30 polypeptide fragments for reducing the insulin resistance and hyperglycaemia in this model either alone or in combination with leptin, the leptin peptide (US provisional application No 60/155,506), or other compounds. Assays include that described previously in Gavrilova et al. ((2000) Diabetes Nov;49(11):1910-6; (2000) Nature Feb 24;403(6772):850) using A-ZIP/F-1 mice, except that gACRP30 would be administered using the methods previously described in Example 5 (or Examples 8-10). The glucose and insulin levels of the mice would be tested, and the food intake and liver weight monitored, as well as other factors, such as leptin, FFA, and TG levels, typically measured in our experiments (see Example 5, above, or Examples 8-10).

EXAMPLE 9: Effect of gACRP30 on plasma Free Fatty Acid in C57 BL/6 Mice

The effect of the globular head of ACRP30 on postprandial lipemia (PPL) in normal C57BL6/J mice was tested. ACRP30 is the mouse protein homologue to the human APM1 protein. The globular head form is indicated by placing a 'g' in front, *i.e.* gACRP30. The gACRP30 used was prepared by proteolytic digestion of recombinant ACRP30 as described previously in Example 2. Acetylated trypsin was used as protease.

The mice used in this experiment were fasted for 2 hours prior to the experiment after which a baseline blood sample was taken. All blood samples were taken from the tail using EDTA coated capillary tubes (50 µL each time point). At time 0 (8:30 AM), a standard high fat meal (6g butter, 6 g sunflower oil, 10 g nonfat dry milk, 10 g sucrose, 12 mL distilled water p repared fresh following Nb#6, JF, pg.1) was given by gavage (vol.=1% of body weight) to all animals.

Immediately following the high fat meal, 25µg gACRP30 was injected i.p. in 100 µL saline. The same dose (25µg/mL in 100µL) was again injected at 45 min a nd at 1 hr 45 min (treated group, n=8). Control animals (n=8) were injected with saline (3x100µL). Untreated and treated animals were handled in an alternating mode.

Blood samples were taken in hourly intervals, and were immediately put on ice. Plasma was prepared by centrifugation following each time point. Plasma was kept at –20°C and free fatty acids (FFA), triglycerides (TG) and glucose were determined within 24 hours using standard test kits (Sigma and Wako). Due to the limited amount of plasma available, glucose was determined in duplicate using pooled samples. For each time point, equal volumes of plasma from all 8 animals per treatment group were pooled. Error bars shown for glucose therefore represent the SD of the duplicate determination an d not the variation between animals as for TG and FFA.

Results

The increase in plasma FFA due to the high fat meal was significantly lower in mice treated with gACRP30 at all time points between 1 and 4 hr. This can be interpreted as increase in FFA oxida tion.

Treatment with gACRP30 also led to a significantly smaller increase in plasma TG compared to untreated mice. However, this effect was less pronounced than the effect on F9.

Glucose turnover was significantly improved following treatment with gACRP3 0; this effect can be interpreted as improved insulin sensitivity possibly due to the decrease in FFA.

Similar results were seen previously in a prior experiment involving only 2 treatments (at 0 and at 45 minutes; data not shown). A strong FFA lowering effect of gACRP30 coupled with a less dominant TG lowering effect was observed.

EXAMPLE 10: Effect of gACRP30 on Plasma Leptin and Insulin in C57 BL/6 Mice

The effect of the globular head of ACRP30 on plasma leptin and insulin levels during postprandial lipemia (PPL) in normal C57BL6/J mice was tested. The experimental procedure was the same as that described in Example 8, except that blood was drawn only at 0, 2 and 4 hours to allow for greater blood samples needed for the determination of leptin and ins ulin by RIA.

Briefly, 16 mice were fasted for 2 hours prior to the experiment after which a baseline blood sample was taken. All blood samples were taken from the tail using EDTA coated capillary tubes (100 µL each time point). At time 0 (9:00AM), a st andard high fat meal (see Example 8) was given by gavage (vol.=1% of body weight) to all animals. Immediately following the high fat meal, 25 µg gACRP30 was injected i.p. in 100 µL saline. The same dose (25µg in 100µL) was again injected at 45 min and at 1 hr 45 min (treated group, n=8). Control animals (n=8) were injected with saline (3x100µL). Untreated and treated animals were handled in an alternating mode.

Blood samples were immediately put on ice and plasma was prepared by centrifugation following each time point. Plasma was kept at –20°C and free fatty acids (FFA) were determined within 24 hours using a standard test kit (Wako). Leptin and Insulin were determined by RIA (ML -82K and SRI-13K, LINCO Research, Inc., St. Charles, MO) following the manufacturer's protocol. However, only 20 µL plasma was used. Each determination was done in duplicate. Due to the limited amount of plasma available, leptin and insulin were determined in 4 pools of 2 animals each in both treatment groups.

Results

As shown previously (Example 8), treatment with gACRP30 significantly reduced the postprandial increase in plasma FFA caused by the high fat meal at 2 hours. There was no significant change in plasma leptin levels at any time point; treatment with gACRP30 did not affect leptin levels. Insulin levels indicate a marginal increase in insulin at 2 hours. However, when analyzed as percentage change from t_0, this increase (212% vs. 260%, control vs. treated) was statistically not significant ($p = 0.09$).

These data reconfirm the previously shown acceleration of FFA metabolism by treatment with gACRP30. They also show that gACRP30 does not affect leptin and insulin plasma levels and that gACRP30 reduces hyperglycemia during postprandial lipemia and also induces weig ht loss during treatment over several days. Without being limited by any particular theory, the data suggests: a) that the reduction in weight is caused by a leptin independent increase in metabolism; and b) that gACRP30 leads to increased insulin sensit ivity.

EXAMPLE 11: Effect of ACRP30 on Plasma FFA, TG and Glucose in C57 BL/6 Mice

The effect of the globular head of ACRP30 on plasma FFA, TG, glucose, leptin and insulin levels during postprandial lipemia (PPL) in normal C57BL6/J mice has been described. Weight loss resulting from gACRP30 (2.5µg/day) given to normal C57BL6/J mice on a high fat diet has also been shown (Example 5). In comparison, a much higher dose of the complete form of ACRP30 (200µg/day) was needed to induce a relatively smaller effect in mice. This example shows the effect of the ACRP30-complete form on plasma FFA, TG and glucose levels.

The experimental procedure was similar to that described in Example 8. Briefly, 14 mice were fasted for 2 hours prior to the experiment after which a baseline blood sample was taken. All blood samples were taken from the tail using EDTA coated capillary tubes (50 µL each time point). At time 0 (9:00AM), a standard high fat meal (see Example 8) was given by gavage (vol.=1% of body weight) to all animals. Immediately following the high fat meal, 4 mice were injected 25 µg ACRP30 i.p. in 100µL saline. The same dose (25µg in 100µL) was again injected at 45 min and at 1 hr 45 min. A second treatment group (n=4) received 3 times 50 µg ACRP30 at the same intervals. Control animals (n=6) were injected with saline (3x100µL). Untreated and treated animals were handled in an alternating mode.

Blood samples were immediately put on ice. Plasma was prepared by centrifugation following each time point. Plasma was kept at –20 °C and free fatty acids (FFA), triglycerides (TG) and glucose were determined within 24 hours using standard test kits (Sigma and Wako).

Results

Treatment with full length ACRP30 had no effect on plasma FFA levels except for t = 2 hours when a statistically significant reduction was shown ($p<0.05$). No significant change in postprandial TG and glucose levels was seen in treated animals.

The data presented show that the complete form of ACRP30 did not reduce FFA, TG and glucose levels in contrast to what was observed for the globular region (Examples 5, 8, 9). Only at 2 hours post-gavage, did treatment with ACRP30 reduce FFA plasma concentrations significantly ($p<0.05$). These results demonstrate that gACRP30 is much more active *in vivo* than the full length protein. A similar effect was seen for body weight reduction; the globular head was much more active than the full-length protein.

EXAMPLE 12: Effect of gACRP30 on FFA following Epinephrine Injection

In mice, plasma free fatty acids increase after intragastric administration of a high fat/sucrose test meal. These free fatty acids are mostly produced by the activity of lipolytic enzymes *i.e.* lipoprotein lipase (LPL) and hepatic lipase (HL). In this species, these enzymes are found in significant amounts

both bound to endothelium and freely circulating in plasma. Another source of plasma free fatty acids is hormone sensitive lipase (HSL) that releases free fatty acids from adipose tissue after β-adrenergic stimulation. To test whether gACRP30 also regulates the metabolism of free fatty acid released by HSL, mice were injected with epinephrine.

Two groups of mice (n=5 each) were given epinephrine (5μg) by intraperitoneal injection. A treated group was injected with gACRP30 (25μg) one hour before and again together with epinephrine, while control animals received saline. Plasma was isolated and free fatty acids and glucose were measured as described above (Example 10). Epinephrine injections (5 μg) caused an increase in plasma free fatty acids and glucose. Both effects were significantly reduced in gACRP30 - treated mice.

This reduction in the increases of glucose and FFA levels was not due to blockage of the β-adrenergic effect of epinephrine, as shown by inducing the release of FFA from isolated adipose tissue *in vitro*. In these control studies, adipose tissue was removed from normal C57BL/6J mice and incubated in Krebs-Henseleit bicarbonate buffer. Epinephrine was added and the concentration of FFA in the medium following a 90 min incubation was determined. Epinephrine (10 μM) caused a 1.7-fold increase in free fatty acids in the media. Increasing concentrations of gACRP30 or ACRP30 up to 50μg/ml did not inhibit this effect of epinephrine.

The data presented thus far indicate that the globular region of ACRP30 exerts profound pharmacological effects on the metabolism of energy substrates with the most evident effect on plasma free fatty acids. Further, the reduction in plasma FFA concentration cannot be explained by inhibition of either LPL – this would cause an increase in plasma triglycerides while a decrease of plasma triglycerides is actually observed – or by inhibition of HSL. Thus, the simplest explanation is that gACRP30 causes increased removal of free fatty acids from the circulation by promoting cellular uptake.

EXAMPLE 13: Effect of gACRP30 on Muscle FFA Oxidation

To investigate the effect of gACRP30 on muscle free fatty acid oxidation, intact hind limb muscles from C57BL/6J mice were isolated and FFA oxidation was measured using oleate as substrate (Clee et al (2000) J Lipid Res 41:521-531; Muoio et al (1999) Am J Physiol 276:E913-921). Oleate oxidation in isolated muscle was measured as previously described (Cuendet et al (1976) J Clin Invest 58:1078-1088; Le Marchand-Brustel (1978) Am J Physiol 234:E348-E358). Briefly, mice were sacrificed by cervical dislocation and soleus and EDL muscles were rapidly isolated from the hind limbs. The distal tendon of each muscle was tied to a piece of suture to facilitate transfer among different media. All incubations were carried out at 30°C in 1.5 mL of Krebs-Henseleit bicarbonate buffer (118.6 mM NaCl, 4.76 mM KCl, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 2.54 mM $CaCl_2$, 25mM $NaHCO_3$, 10 mM Hepes, pH 7.4) supplemented with 4% FFA free bovine serum albumin (fraction V, RIA grade, Sigma)

and 5 mM glucose (Sigma). The total concentration of oleate (Sigma) throughout the experiment was 0.25 mM. All media were oxygenated (95% O_2; 5% CO_2) prior to incubation. The gas mixture was hydrated throughout the experiment by bubbling through a gas washer (Kontes Inc., Vineland, NJ).

Muscles were rinsed for 30 min in incubation media with oxygenation. The muscles were then transferred to fresh media (1.5 mL) and incubated at 30°C in the presence of 1μCi/mL [1-^{14}C] oleic acid (American Radiolabeled Chemicals). The incubation vials containing this media were sealed with a rubber septum from which a center well carrying a piece of Whatman paper (1.5 cm x 11.5 cm) was suspended.

After an initial incubation period of 10min with constant oxygenation, gas circulation was removed to close the system to the outside environment and the muscles were incubated for 90 min at 30°C. At the end of this period, 0.45 mL of Solvable (Packard Instruments, Meriden, CT) was injected onto the Whatman paper in the center well and oleate oxidation by the muscle was stopped by transferring the vial onto ice.

After 5 min, the muscle was removed from the medium, and an aliquot of 0.5 mL medium was also removed. The vials were closed again and 1 mL of 35% perchloric acid was injected with a syringe into the media by piercing through the rubber septum. The CO_2 released from the acidified media was collected by the Solvable in the center well. After a 90 min collection period at 30°C, the Whatman paper was removed from the center well and placed in scintillation vials containing 15 mL of scintillation fluid (HionicFlour, Packard Instruments, Meriden, CT). The amount of ^{14}C radioactivity was quantitated by liquid scintillation counting. The rate of oleate oxidation was expressed as nmol oleate produced in 90min/g muscle.

To test the effect of gACRP30 or ACRP30 on oleate oxidation, these proteins were added to the media at a final concentration of 2.5 μg/mL and maintained in the media throughout the procedure.

Two muscles of different oxidative capacity [soleus and extensor digitorum longus (EDL)] were tested. EDL and Soleus muscles were isolated from both legs of normal C57BL/6J mice (n=18). One muscle of each pair was incubated in medium with 2.5 μg/mL gACRP30 (dark gray) and one in medium without gACRP30 (control – light gray). This experimental design allowed us to compare oleate oxidation in pairs of muscles isolated from the same animal. ^{14}C-Oleate oxidation was determined over 90 minutes. Incubation of EDL and soleus muscles for 90 minutes in medium containing 2.5μg/ml gACRP30 leads to a statistically significant increase in oleate oxidation ($p<0.05$, paired, one-tailed, t-Test) or ($p=0.0041$, Repeated Measures Analysis of Variance, Univariate Tests of Hypotheses for Within Subject Effects) in both muscle types.

Both muscle types showed a significant response to gACRP30. The relative increase in FFA oxidation was 17% ($p=0.03$) and 10% ($p=0.04$) for EDL and soleus, respectively. In humans, muscles

represent approximately 25% of body weight. Therefore, even a moderate increase in free fatty acid oxidation can have quantitatively important consequences on overall energy utilization.

EXAMPLE 14: Effect of gArcp30 on Triglyceride in Muscle & Liver Isolated from Mice

To determine whether the increased FFA oxidation induced by gACRP30 is also accompanied by increased FFA delivery into muscle or liver, the hindlimb muscle and liver triglyceride content was measured after gACRP30 treatment of mice. Hind limb muscles as well as liver samples were removed from treated and untreated animals and the triglyceride and free fatty acid concentration was determined following a standard lipid extraction method (Shimabukuro et al (1997) Proc Natl Acad Sci USA 94:4637-4641) followed by TG and FFA analysis using standard test kits.

Short-term treatment of animals with gACRP30 (2 injections of 25 µg each given within 3 hours before sacrifice) did not change the triglyceride content either of hind limb muscle or liver tissue (data not shown). However, after 3 days of treatment, during which period normal C57BL/6J mice consumed a regular rodent diet, mice that had received 25 µg of gACRP30 twice daily showed significantly higher ($p=0.002$) muscle triglyceride content than those receiving saline (control: light gray; gACRP30: dark gray). This contrasted with a lack of increase in liver triglycerides. Furthermore, no detectable increase in muscle TG was observed after the 16-day treatment shown independently by directly measuring the muscle TG content and by oil red O staining of frozen microscope sections. In summary, the data indicate that the increase in TG content was transient.

These data are consistent with the notion that gACRP30 increases the rate of removal of free fatty acids from plasma at least partly by increasing their delivery to the muscle; much of the FFAs are immediately oxidized while some are stored as triglycerides and subsequently oxidized. Further support for this interpretation was obtained by measuring the concentration of ketone bodies in plasma of treated and untreated animals following a high fat/sucrose meal.

Ketone bodies (KB) are produced in the liver as a result of free fatty acid oxidation, but KB formation does not occur significantly in muscle. In mice receiving the high fat test meal and saline injection, the level of plasma KB increased significantly over the next 3 hours (183 ± 12%, n=6). Animals treated with gACRP30, on the other hand, showed no increase in plasma KB concentrations. Thus, gACRP30 inhibits either directly KB formation or can decrease KB production by inhibiting liver FFA oxidation.

EXAMPLE 15: Effect of gACRP30 on Weight Gain & Weight Loss of Mice

Two independent studies showed that gACRP30 also affects overall energy homeostasis. In the first, 10-week-old male C57BL/6J mice were put on a very high fat/sucrose purified diet for 19 days to

promote weight gain (see Example 5); the average body weight at this time was 30g. The mice were then surgically implanted with an osmotic pump (Alzet, Newark, DE) delivering either 2.5 µg/day of gACRP30, 5µg/day of ACRP30, or physiological saline. The mice were continued on the high fat diet and their body weight was recorded over the following 10-day period.

Mice treated with saline or 5 µg/day of full length ACRP30 continued to gain weight at an average daily rate of 0.16% and 0.22%, respectively. In contrast, mice treated with gACRP30 experienced a significant weight reduction (-3.7%, $p = 0.002$) during the first 4 days and then their weight remained constant. Thus, in this inbred strain of normal mice, a continuous infusion of a daily low dose of gACRP30 can prevent weight gain caused by high fat/sucrose feeding, in a sustainable way.

This result was confirmed and extended in a second study performed in mature 9 month old, male obese C57BL/6J mice that had been on the same high fat/sucrose diet for 6 months; the average body weight when the study began was 52.5±0.8g. Three groups of 8 mice were treated with saline, ACRP30 or gACRP30 for 16 days. Animals in the treated group received twice daily 25 µg of protein subcutaneously. Body weights were recorded at the indicated time points.

Treatment with gACRP30 led to significant ($p<0.05$) weight loss at day 3. This effect became even more significant as the study continued. During the 16 day study period, the obese C57BL/6J mice that received gACRP30 lost about 8% ($p=0.001$) of their initial body weight despite the fact that they were maintained on a high fat/sucrose diet. Saline treated animals showed only marginal fluctuations in their body weight (p = n.s.). Animals treated with the full length ACRP30, but at a 10-fold higher dose than that used in the first experiment, also lost significant weight (-3.2%, $p=0.025$). Interestingly, mice treated with gACRP30 continued to lose weight at a steady rate during the 16-day study period, while the rate of weight reduction in those treated with the full length ACRP30 decreased during the later phase of the study. Food consumption in gACRP30 treated animals was not significantly different from saline or ACRP30 treated animals.

Treatment with gACRP30 caused a significant reduction in the concentration of plasma free fatty acids. This effect was significant after 3 days of treatment ($p<0.05$ vs. saline) and continued throughout the complete study period. Shown is the plasma FFA level at day 16 of the study. The initial FFA plasma concentration was the same in all three treatment groups. It should be noted, however, that despite this reduction the plasma free fatty acid concentration of these massively obese animals remains about 40-60% higher than that of normal mice. A blood chemistry analysis (including determination of SGPT, SGOT, urea, creatinine or bilirubin) performed on the terminal blood samples did not reveal any abnormal plasma parameters.

Data are expressed throughout as mean ± SEM; a p-value < 0.05 was considered statistically significant. Statistical analysis was typically done using either the unpaired Student's t test or the paired Student's t test, as indicated in each study.

EXAMPLE 16: Detection of APM1 (gACRP30) Fragment in Human Plasma After Immunoprecipitation

The recombinant form of ACRP30 protein used has an apparent molecular weight of 37 kDa and forms a dimer of 74 kDa. A proteolytic fragment that contains the entire globular head region (gACRP30) and that migrates with an apparent molecular weight of 18 kDa was generated using acetylated trypsin. Both protein preparations (ACRP30 and gACRP30) were essentially endotoxin free; ActiClean Etox affinity columns (Sterogene Bioseparations Inc., Carlsbad, CA) were used to remove potential endotoxin contaminations following the manufacturer's protocol. Endotoxin levels were determined by Endosafe, Charleston, SC. As determined by N-terminal sequencing of purified gACRP30, the site of cleavage was just before amino acid 104 (just before amino acid 101 for human gACRP30 or APM1).

Immunoprecipitation of human plasma APM1 followed by Western blotting was used to detect a cleavage product of APM1, the human homolog of ACRP30, using a globular head specific anti-serum for the immunoprecipitation step as well as for the detection step. Preimmune serum or serum raised against the globular head domain or human non-homologous region (HDQETTTQGPGVLLPLPKGA) were cross-linked to protein A (Sigma Chemical CO, Saint Louis, MO) using dimethyl-pimelimidate-dihydrochloride (Sigma Chemical Co, Saint Louis, MO). After washing (0.2 M salt) proteins were eluted from protein A, separated by SDS-PAGE, transferred to Protran® pure nitrocellulose membrane (Schleicher and Schuell, Keene, NH) using standard procedures. APM1 products were visualized using globular head domain antibodies labeled with biotin; horseradish peroxidase conjugated to Streptavidin and CN/DAB substrate kit (Pierce, Rockford, IL) according to manufacturer's instructions.

The apparent molecular weight of this truncated form was 27 kDa, corresponding to about 70% of the complete form of APM1. This truncated form was not detectable when immunoprecipitation was performed using a different antibody directed against the human non-homologous region (HDQETTTQGPGVLLPLPKGA) of APM1; this domain is located toward the NH_2 terminal end of the protein outside of the globular domain. Both anti-APM1 antibodies directed against either the globular or the non-globular domain identified the full-length form of the protein, as well as a low abundance dimer of apparent MW 74 kDa.

EXAMPLE 17: Effect of gACRP30 on FFA following Intralipid Injection

Two groups of mice (n=5 each) were intravenously (tail vein) injected with 30 µL bolus of Intralipid-20% (Clintec) to generate a sudden rise in plasma FFAs, thus by-passing intestinal absorption. (Intralipid is an intravenous fat emulsion used in nutritional therapy). A treated group was injected with

gACRP30 (25μg) at 30 and 60 minutes before Intralipid was given, while control animals received saline. Plasma was isolated and FFAs were measured as described previously.

The effect of gACRP30 on the decay in plasma FFAs following the peak induced by Intralipid injection was then monitored. gACRP30 accelerates the removal of FFAs from plasma after Intralipid injection. Thus, gACRP30 accelerates the clearance of FFAs without interfering with intestinal absorption. Although not wishing to be bound by any theory, because Intralipid does not elicit a significant insulin response, the results also indicate that gACRP30 regulation of FFA metabolism occurs independently of insulin.

EXAMPLE 18: Use of Biacore Technology to Detect Specific Binding of gAPM1 Polypeptide Fragment to TNFRSF2 Polypeptide Fragment

Biacore utilizes a biosensor technology for monitoring interactions between two or more molecules in real time, without the use of labels. The molecular classes than can be studied are diverse, ranging from proteins, peptides, nucleic acids, carbohydrates, and lipids to low molecular weight substances and pharmaceuticals. Molecules do not need to be purified or even solubilized, but can be studied in crude extracts, serum, anchored on lipid vesicles, and in some cases viruses and eucaryotic cells.

The detection principle is based on the optical phenomena of surface plasmon resonance, which detects changes in refractive index close to a biosensor surface. In a typical experiment one of the interacting molecules is immobilized or captured (ligand) to a flexible dextran layer close to the sensor surface. The interacting partner (analyte) is flowed across that surface. If an interaction occurs between the two molecules, there is a resulting increase in signal due to the increase in mass at the chip surface. The signal is recorded in resonance units. Questions that can be addressed using Biacore technology relate to specifity (which components interact and under what conditions), kinetics (how fast do the components bind and dissociate), affinity (what is the strength of the binding), and concentration (how much active component is present in a particular sample).

Biacore technology as described herein was used to determine whether gAPM1 polypeptide fragment binds specifically to the extracellular domain of TNFRSF2 polypeptide.

Soluble TNFRSF2 extracellular domain was attached to the sensor surface via amine coupling chemistry. The dextran was activated using N-hydroxysuccinimide and N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride for 7 minutes. Soluble TNFRSF2 extracellular domain was diluted in 10mM Na Acetate pH 5.0 at a concentration of 10 μg/ml and injected over the activated surface for 7 minutes. The surface was then blocked for 7 minutes using ethanolamine to remove any remaining esters. A blank flow cell absent soluble TNFRSF2 extracellular domain was set

up in parallel and used as a control surface. The running buffer was HBS-EP (0.01M HEPES pH 7.4, 0.15M NaCl, 3mM EDTA, 0.005% Surfactant P20) and the instrument temperature was 25 °C.

The gAPM1 polypeptide fragment was filtered through an Ultrafree-0.5 Centrifugal Filter Device and resuspended in HBS-EP running buffer. The protein was then diluted 1:10 in HBS-EP and injected over the soluble TNFRSF2 extracellular domain surface and the blank control surface for 1 minute at a flow rate of 50 µl/min. The sensorgrams from the receptor surface and the control surface were aligned and overlayed. No binding was observed on the control surface.

To obtain the specific binding, the control surface was subtracted from the active surface comprised of soluble TNFRSF2 extracellular domain.

To show binding specificity, the gAPM1 polypeptide fragment was injected at various dilutions over the soluble TNFRSF2 extracellular domain surface and the control surface. The sensorgrams from each dilution were overlayed and subtracted as before.

The binding of gAPM1 polypeptide fragment to soluble TNFRSF2 extracellular domain on the surface was partially blocked with soluble TNFRSF2 extracellular domain. Soluble TNFRSF2 extracellular domain was added to the gAPM1 polypeptide fragment dilutions and the mixture was then injected over the soluble TNFRSF2 extracellular domain surface and control surface. The sensorgrams from each dilution were overlayed and subtracted as before.

In summary gAPM1 polypeptide fragment bound to the soluble TNFRSF2 extracellular doma in. The apparent association and dissociation rates of the gAPM1 polypeptide fragment binding to the soluble TNFRSF2 extracellular domain appeared to be very slow suggesting that the binding is a high affinity interaction.

Significantly, analogous experiments using soluble Interferon-gamma Receptor (IFNgamma R1), Fibroblast Growth Factor Receptor 4 (FGF R4), or Leptin Receptor extracellular domain failed to detect binding of gAPM1 polypeptide fragment, in contrast to what was observed for soluble TNFRSF2 extracellular domain.

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Throughout this application, various publications, patents and published patent applications are cited. The disclosures of these publications, patents and published patent specification referenced in this application are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

TABLE 1.1

Amino Acid Residues Comprising the Structural Domains of FRADJ

SEQ ID NO: 2 Description

SIGNAL PEPTIDE	EC DOMAIN	TRANSMEMBRANE DOMAIN	IC DOMAIN
1-27	28-78	79-101	102-129

EC, extracellular domain; IC, intracellular domain

TABLE 1.2

Amino Acid Residues Comprising the Structural Domains of CRYPTIC

SEQ ID NO: 4 Description

SIGNAL PEPTIDE	EC DOMAIN	TRANSMEMBRANE DOMAIN	IC DOMAIN
1-27	28-43	44-66	67-94

EC, extracellular domain; IC, intracellular domain

TABLE 2

LIGANDS of FRADJ and/or CRYPTIC

>APM1 polypeptide sequence:

MLLLGAVLLLLALPGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDGTPG
EKGEKGDPGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGAYV YRSAFSVGLETYVTIPNM
PIRFTKIFYNQQNHYDGSTGKFHCNIPGLYYFAYHITVYMKDVKVSLFKKDKAMLFTYDQYQEN
NVDQASGSVLLHLEVGDQVWLQVYGEGERNGLYADNDNDSTFTGFLLYHDTN (244)

>ACRP30 polypeptide sequence:

MLLLQALLFLLILPSHAEDDVTTTEELAPALVPPPKGTCAGWMAGIPGHPGHNGTPGRDGRDGT
PGEKGEKGDAGLLGPKGETGDVGMTGAEGPRGFPGTPGRKGEPGEAAYM YRSAFSVGLETRVT
VPNVPIRFTKIFYNQQNHYDGSTGKFYCNIPGLYYFSYHITVYMKDVKVSLFKKDKAVLFTYDQ
YQEKNVDQASGSVLLHLEVGDQVWLQV YGDGDHNGLYADNVNDSTFTGFLLYHDTN (247)

> C2P polypeptide sequence:

MRIWWLLLAIEICTGNINSQDTCRQGHPGIPGNPGHNGLPGRDGRDGAKGDKGDAGEPGRPGSP
GKDGTSGEKGERGADGKVEAKGIKGDQGSRGSPGKHGPKGLAGPMGEKGLRGETGPQGQKGN
KGDVGPTGPEGPRGNIGPLGPTGLPGPMGPIGKPGPKGEAGPTGPQGEPGVRGIRGWKGDRGE K
GKIGETLVLPKSAFTVGLTVLSKFPSSDMPIKFDKILYNEFNHYDTAAGKFTCHIAGVYYFTYHIT
VFSRNVQVSLVKNGVKILHTKDAYMSSEDQASGGIVLQLKLGDEVWLQVTGGERFNGLFADED
DDTTFTGFLLFSSP (333)

> D2P polypeptide sequence:

MRIWWLLLAIEICTGNINSQDTCRQGHPGIPGNPGHNGLPGRDGRDGAKGDKGDAGEPGCPGSP
GKDGTSGEKGERGADGKVEAKGIKGDQGSRGSPGKHGPKGLAGPMGEKGLRGETGPQGQKGN
KGDVGPTGPEGPRGNIGPLGPTGLPGPMGPIGKPGPKGEAGPTGPQGEPGVRGIRGWKGDRGEK
GKIGETLVLPKSAFTVGLTVLSKFPSSDVPIKFDKILYNEFNHYDTAVGKFTCHIAGVYYFTYHIT
VFSRNVQVSLVKNGVKILHTRDAYVSSEDQASGSIVLQLKLGDEMWLQVTGGERFNGLFADED
DDTTFTGFLLFSSQ (333)

>ACRP30R1 polypeptide sequence:

MIPWVLLACALPCAADPLLGAFARRDFRKGSPQLVCSLPGPQGPPGPPGAPGPSGMMGRMGFP
GKDGQDGHDGDRGDSGEEGPPGRTVTKSYPRERLPIKFDKILMNEGGHYNASSGKFVCGVPGIY
YFTYDITLANKHLAIGLVHNGQYRIRTFDANTGNHDVASGSTILALKQGDEVWLQIFYSEQNGL
FYDPYWTDSLFTGFLIYADQDDPNEV (217)

>ACRP30R1L polypeptide sequence:

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GKDGQDGHDGDRGDSGEEGPPGRTGNRGKPGPKGKAGAIGRAGPRGPKGVNGTPGKHGTPGK
KGPKGKKGEPGLPGPCSCGSGHTKSAFSVAVTKSYPRERLPIKFDKILMNEGGHYNASSGKFVC
GVPGIYYFTYDITLANKHLAIGLVHNGQYRIRTFDANTGNHDVASGSTILALKQGDEVWLQIFYS
EQNGLFYDPYWTDSLFTGFLIYADQDDPNEV (285)

>HGS polypeptide sequence

MPRVRKEPEAMQWLRVRESPGEATGHRVTMGTAALGPVWAALLLFLLMCEIPMVELTFDRAV
ASDCQRCCDSEDPLDPAHVSSASSSGRPHALPEIRPYINITILKGDKGDPGPMGLPGYMGREGPQ
GEPGPQGSKGDKGEMGSPGAPCQKRFFAFSVGRKTALHSGEDFQTLLFERVFVNLDGCFDMAT
GQFAAPLRGIYFFSLNVHSWNYKETYVHIMHNQKEAVILYAQPSERSIMQSQSVMLDLAYGDR
VWVRLFKRQRENAIYSNDFDTYITFSGHLIKAEDD (288)

>ZACRP6 polypeptide sequence:

MQWLRVRESPGEATGHRVTMGTAALGPVWAALLLFLLMCEIPMVELTFDRAVASGCQRCCDS
EDPLDPAHVSSASSSGRPHALPEIRPYINITILKGDKGDPGPMGLPGYMGREGPQGEPGPQGSKG
DKGEMGSPGAPCQKRFFAFSVGRKTALHSGEDFQTLLFERVFVNLDGCFDMATGQFAAPLRGIY
FFSLNVHSWNYKETYVHIMHNQKEAVILYAQPSERSIMQSQSVMLDLAYGDRVWVRLFKRQRE
NAIYSNDFDTYITFSGHLIKAEDD (278)

>ACRP30R2 polypeptide sequence:

MGTAALGPVWAALLLFLLMCEIPMVELTFDRAVASGCQRCCDSEDPLDPAHVSSASSSGRPHAL
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GRKTALHSGEDFQTLLFERVFVNLDGCFDMATGQFAAPLRGIYFFSLNVHSWNYKETYVHIMH
NQKEAVILYAQPSERSIMQSQSVMLDLAYGDRVWVRLFKRQRENAIYSNDFDTYITFSGHLIKA
EDD (259)

>ZACRP5 polypeptide sequence:

MAAPALLLLALLLPVGAWPGLPRRPCVHCCRPAWPPGPYARVSDRDLWRGDLWRGLPRVRPTI
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LHSSDHFQAVPFDTELVNLDGAFDLAAGRFLCTVPGVYFLSLNVHTWNYKETYLHIMLNRRPA
AVLYAQPSERSVMQAQSLMLLLAAGDAVWVRMFQRDRDNAIYGEHGDLYITFSGHLVKPAAE
L (252)

>ZSIG37 polypeptide sequence:

MGSRGQGLLLAYCLLLAFASGLVLSRVPHVQGEQQEWEGTEELPSPPDHAERAEEQHEKYRPS
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GERENAIFSEELDTYITFSGYLVKHATEP (281)

>ZACRP4 polypeptide sequence:

MLPLLLGLLGPAACWALGPTPGPGSS ELRSAFSAARTTPLEGTSEMAVTFDKVYVNIGGDFDVA
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YGDTVWLRLHGAPHYALGAPGATFSGYLVYADADADAP ARGPPAPPEPRSAFSAARTRSLVGS
DAGPGPRHQPLAFDTEFVNIGGDFDAAAGVFRCRLPGAYFFSFTLGKLPRKTLSVKLMKNRDEV
QAMIYDDGASRRREMQSQSVMLALRRGDAVWLLSHDHDGYGAYSNHGKYITFSGFLVYPDLA
PAAPPGLGASELL (329)

>ZSIG39 polypeptide sequence:

MRPLLVLLLLGLAAGSPPLDDNKIPSLCPGHPGLPGTPGHHGSQGLPGRDGRDGRDGAPGAPGE
KGEGGRPGLPGPRGDPGPRGEAGPAGPTGPAGECSVPPRSAFSAKRSESRVPPPSDAPLPFDRVL
VNEQGHYDAVTGKFTCQVPGVYYFAVHATVYRASLQFDLVKNGESIASFFQFFGGWPKPASLS
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>ZACRP3 polypeptide sequence:

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GNHGNNGNNGATGHEGAKGEKGDKGDLGPRGERGQHGPKGEKGYPGIPPELQIAFMASLATHF
SNQNSGIIFSSVETNIGNFFDVMTGRFGAPVSGVYFFTFSMMKHEDVEEVYVYLMHNGNTVFSM
YSYEMKGKSDTSSNHAVLKLAKGDEVWLRMGNGALHGDHQRFSTFAGFLLFETK (246)

>ZACRP7 polypeptide sequence:

MGKEDTQETRTEPKMFVLLYVTSFAICASGQPRGNQLKGENYSPRYICSIPGLPGPPGPPGANGS
PGPHGRIGLPGRDGRDGRKGEKGEKGTAGLRGKTGPLGLAGEKGDQGETGKKGPIGPEGEKGE
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LEIFFTDQNGLFSDPGWADSLFSGFLLYVDTDYLDSISEDDEL (303)

>C1RF-3 polypeptide sequence:

MALGLLIAVPLLLQAAPPGAAHYEMLGTCRMICDPYSVAPAGGPAGAKAPPPGPSTAALEVMQ
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GKFSCQVRGIYFFTYHILMRGGDGTSMWADLCKNGQVRASAIAQDADQNYDYASNSVVLHLD
SGDEVYVKLDGGKAHGGNNNKYSTFSGFLLYPD (287)

>C1RF-2 polypeptide sequence:

MVLLLVILIPVLVSSAGTSAHYEMLGTCRMVCDPYGGTKAPSTAATPDRGLMQSLPTFIQGPKG
EAGRPGKAGPRGPPGEPGPPGPMGPPGEKGEPGRQGLPGPPGAPGLNAAGAISAATYSTGPKIAF
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KNNQVRASAIAQDADQNYDYASNSVVLHLEPGDEVYIKLDGGKAHGGNNNKYSTFSGFIIYAD
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>C1RF-1 polypeptide sequence:

MLLVLVVLIPVLVSSGGPEGHYEMLGTCRMVCDPYPARGPGAGARTDGGDALSEQSGAPPPSTL
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YSD (258)

CLAIMS

1. A method of using a polypeptide comprising SEQ ID NO: 2 (FRADJ), or a fragment thereof, to screen for antagonists or agonists of APM1 polypeptide or polypeptide fragment activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity.

2. A method of using a polypeptide comprising SEQ ID NO: 4 (CRYPTIC), or a fragment thereof, to screen for antagonists or agonists of APM1 polypeptide or polypeptide fragment activity, wherein said activity is selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity.

3. A method for determining whether a ligand can bind specifically to FRADJ, or a fragment therof, which comprises (a) contacting FRADJ, or the fragment thereof, with the ligand under conditions permitting binding of a ligand to FRADJ, or the fragment thereof, and (b) detecting the presence of the ligand bound specifically to FRADJ, or the fragment thereof.

4. The method according to claim 3, wherein the ligand is an antagonist of FRADJ.

5. The method according to claim 3, wherein the ligand is an agonist of FRADJ.

6. A method for determining whether a ligand can bind specifically to CRYPTIC, or a fragment therof, which comprises (a) contacting CRYPTIC, or the fragment thereof, with the ligand under conditions permitting binding of a ligand to CRYPTIC, or the fragment thereof, and (b) detecting the presence of the ligand bound specifically to CRYPTIC, or the fragment thereof.

7. The method according to claim 6, wherein the ligand is an antagonist of CRYPTIC.

8. The method according to claim 6, wherein the ligand is an agonist of CRYPTIC.

9. A method of screening chemical compounds to identify drug candidates which specifically bind to FRADJ, or a fragment thereof, comprising (a) contacting FRADJ, or a fragment thereof, with a plurality of compounds, and (b) determining those compounds which bind to FRADJ, or the fragment thereof.

10. The method according to claim 9, wherein the drug candidate is an antagonist of FRADJ.

11. The method according to claim 9, wherein the drug candidate is an agonist of FRADJ.

12. A method of screening chemical compounds to identify drug candidates which specifically bind to CRYPTIC, or a fragment thereof, comprising (a) contacting CRYPTIC, or a fragment thereof, with a plurality of compounds, and (b) determining those compounds which bind to CRYPTIC, or the frag ment thereof.

13. The method according to claim 12, wherein the drug candidate is an antagonist of CRYPTIC.

14. The method according to claim 12, wherein the drug candidate is an agonist of CRYPTIC.

15. Use of an antagonist of FRADJ, or a fragment thereof, for preparation of a medicament for treatment and/or prevention of a disorder associated with excessive weight loss.

16. Use of an antagonist of CRYPTIC, or a fragment thereof, for preparation of a medicament for treatment and/or prevention of a disorder associated with excessive weight loss.

17. Use according to claim 15 or 16, wherein the disorder associated with excessive weight loss is selcected from cachexia, cancer-related weight loss, AIDS-related weight loss, chronic inflammatory disease-related weight loss, bulimia and anorexia.

18. Use of an agonist of FRADJ, or a fragment thereof, for preparation of a medicament for treatment and/or prevention of an obesity-related disorder.

19. Use of an agonist of CRYPTIC, or a fragment thereof, for preparation of a medicament for treatment and/or prevention of an obesity-related disorder.

20. Use according to claim 18 or 19, wherein the obesity-related disorder is selected from obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, syndrome x, noninsulin dependent diabetes mellitus (NIDDM, or type II diabetes), insulin dependent diabetes mellitus (IDDM or type I diabetes),or diabetes-related complications, hyperlipidemia and hyperuricemia.

21. Use according to claim 20, wherein the diabetes-related complication is selected from microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions.

SEQUENCE LISTING

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Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile Gln
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/50066

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 G01N33/50 A61P3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K G01N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 03 011325 A (GENSET SA ;DIALYNAS DENO (US); BRIGGS KRISTEN (US); LUCAS JOHN (US) 13 February 2003 (2003-02-13) the whole document ---	1-14
X	WO 99 59618 A (SMITHKLINE BEECHAM CORP) 25 November 1999 (1999-11-25) the whole document ---	1-14
X	BODMER J-L ET AL: "The molecular architecture of the TNF superfamily" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 27, no. 1, 1 January 2002 (2002-01-01), pages 19-26, XP004332356 ISSN: 0968-0004 the whole document ---	1-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 August 2003	12/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Keller, Y

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/50066

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 68380 A (INCYTE GENOMICS INC ;AZIMZAI YALDA (US); YUE HENRY (US); BANDMAN O) 16 November 2000 (2000-11-16)	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/50066

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 15-21
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 15-21

Present claims15-21 relate to the "use of an agonist/antagonist" defined by reference to a desirable characteristic or property. The claims cover all products/methods having this characteristic or property (i.e being an agonist/antagonist), whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only no such products/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method/apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for these claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/50066

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03011325 A	13-02-2003	WO 03011325 A1	13-02-2003
WO 9959618 A	25-11-1999	EP 1085897 A1	28-03-2001
		EP 1083923 A1	21-03-2001
		JP 2002515452 T	28-05-2002
		WO 9959618 A1	25-11-1999
		WO 9959619 A1	25-11-1999
		US 2001009905 A1	26-07-2001
WO 0068380 A	16-11-2000	AU 5129800 A	21-11-2000
		CA 2372815 A1	16-11-2000
		EP 1177296 A2	06-02-2002
		JP 2002543785 T	24-12-2002
		WO 0068380 A2	16-11-2000